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Effects of Increasing Energy and Protein Intake on Body Growth and and Mammary Development in Holstein Heifer Calves

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EFFECTS OF INCREASING ENERGY AND PROTEIN INTAKE ON BODY GROWTH AND MAMMARY DEVELOPMENT IN HOLSTEIN HEIFER CALVES

By

Erin Gwen Brown

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ABSTRACT

EFFECTS OF INCREASING ENERGY AND PROTEIN INTAKE ON BODY GROWTH AND MAMMARY DEVELOPMENT IN HOLSTEIN HEIFER CALVES

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The objective was to determine if increasing energy and protein intake in heifer calves less than 4 months of age would alter body composition or mammary development. In a 2×2 factorial arrangement of diet and age, Holstein heifer calves (n=53) were fed diets for low (L) or high (H) gains from 2 to 8 weeks and from 8 to 14 weeks of age. The L calves were fed milk replacer at 1.25% of body weight on a dry matter basis (21.3% CP, 21.3% fat) and calf starter (20.5% CP) for body weight gains of 0.4 kg/d from 2 to 8 weeks and 0.43 kg/d from 8 to 14 weeks of age. The high calves were fed milk replacer at 2.25% of body weight on a dry matter basis (30.3% CP, 15.9% fat) and calf starter (25.0% CP) for body weight gains of 0.66 kg/d from 2 to 8 weeks and 1.1 kg/d from 8 to 14 weeks of age. Calves were weaned at 7 weeks of age and were slaughtered at 14 weeks of age. Calves on the H diet from 2 to 8 weeks of age had increased mammary parenchymal mass and increased mammary parenchymal DNA. In conclusion, increasing protein and energy intake in heifer calves from 2 to 8 weeks of age increases mammary development.

This thesis is dedicated to my parents, Gene and Dominga Brown, and my brothers, Gene and Everett, for all your patience and support.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

Abbreviation	Definition
ADG	average daily gain
bST	bovine somatotropin
BW	body weight
CP	crude protein
d	day
DM	dry matter
h	hour
IGF-I	insulin-like growth factor-I
IgG	immunoglobulin G
IU	international units
kg	kilogram
L	liter
min	minute
ml	milliliter
mm	millimeter
ng	nanogram
ppm	parts per million
s	second
wk	week

INTRODUCTION

The cost of raising replacement heifers accounts for approximately 20% of dairy farm expenses (Heinrichs, 1993). One possible way to decrease this cost is to calve heifers at a younger age. To decrease age at first calving, heifers can be fed for accelerated growth rates prior to puberty and bred at an earlier age. Using this strategy, it is possible to raise heifers to a desirable body weight at first calving (600 kg; Heinrichs, 1993) as early as 19 to 20 months of age. However, accelerated rates of gain can impair mammary development and cause excess fat deposition, possibly resulting in problems around the time of calving. By decreasing age at first calving, there is potential to increase lifetime profitability, assuming no negative effects due to accelerated growth.

From birth until approximately 3 months of age, the mammary gland and body grow at similar rates, which is known as isometric growth. After 3 months of age until puberty (7 to 10 months of age), the gland increases in size at a rate 3 times faster than the body, which is referred to as allometric growth (Sinha and Tucker, 1969). After puberty, mammary growth again becomes isometric with body weight gain until pregnancy. High energy diets during the allometric growth period can decrease mammary development and future milk production (Swanson, 1960; Sejrsen et al., 1982; Little and Kay, 1979). However, mammary growth during the post-pubertal isometric period is not affected by diet (Sejrsen et al., 1982). Perhaps growing heifer calves fast (>0.8 kg/d ADG) during the first

period of isometric growth from birth to 3 months of age might not impair mammary development.

Previous research has shown that both increased growth rates and diet changes in young calves alter body composition. Donnelly and Hutton (1976) showed that increasing protein in milk replacer resulted in an increase in carcass protein and a decrease in carcass fat. Increasing fat in milk replacer of calves on an isocaloric, isonitrogenous diet resulted in an increase in carcass fat percentage without altering the carcass protein (Tikofsky et al., 2001). These two studies indicate that a higher protein and lower fat percentage in milk replacer are beneficial in promoting lean tissue deposition.

Possible mechanisms for the effects of diet on mammary development include the somatotropin-IGF-I axis and leptin. Because exogenous somatotropin stimulates mammary growth in dairy heifers (Sejrsen et al., 1986), decreased serum somatotropin concentrations in heifers on a high feeding level have been implicated in the negative effects of a high rate of gain on mammary growth (Sejrsen and Purup, 1997). However, the bovine mammary gland lacks specific receptors for bovine somatotropin (Akers, 1985), indicating an indirect mechanism of action which may involve IGF-I. Heifers receiving exogenous injections of somatotropin also had higher serum IGF-I concentrations than control animals (Sejrsen et al., 1986). Because IGF-I is a potent mitogen for mammary epithelial cells, IGF-I in mammary tissue likely plays an important role in prepubertal mammary development. Further, Silva et al. (2002) found that leptin, a protein produced by adipocytes, decreases IGF-I induced bovine

mammary cell proliferation in vitro. Taken together, these studies indicate a potential role for IGF-I and leptin in mediating the effects of diet on mammary development.

Objectives

The purpose of this thesis research was to determine the effects of increased energy and protein intake in Holstein heifer calves on mammary gland development. The study was a 2×2 factorial design consisting of two dietary regimes (low, L; high, H) and two periods of growth (2 to 8 wk and 8 to 14 wk of age). The objective was to determine if increasing energy and protein intake would alter growth and mammary development in Holstein heifer calves.

LITERATURE REVIEW

The cost of raising dairy heifers constitutes approximately 20% of farm expenses (Heinrichs, 1993). One way to decrease the cost of raising replacement heifers is for the animal to be bred to calve earlier. The onset of puberty in cattle is determined not by age, but instead by body weight (Hall et al., 1995). For a heifer to reach the appropriate body weight for breeding at a younger age, she must be fed for higher rates of gain. However, accelerated rates of gain prior to puberty are known to decrease mammary development and future milk production (Sejrsen et al., 1982, Little and Kay, 1979).

Since the mid-1940's, researchers (Herman and Ragsdale, 1946) have been struggling with the question of how to grow heifers at faster rates of gain without impairing mammary gland development. Heifers started on a rapid growth diet at four months of age produced less milk during their first lactation than heifers started at seven months of age (Swanson, 1960). Sinha and Tucker (1969) concluded that from around three months of age until puberty, the mammary gland grows 3.5 times faster than the body. Most research (Radcliff et al., 2000; Capuco et al, 1995; Sejrsen et al., 1982; Little and Kay,1979) showed that feeding for gains exceeding 0.8 kg/d during this period resulted in more adipose deposition in the mammary gland, less mammary parenchymal tissue and 10-50% lower milk production. After puberty, high rates of gain do not impair mammary gland development (Sejrsen et al, 1982).

High rates of gain prior to 4 months of age have resulted in higher first lactation milk production and similar amounts of mammary parenchymal tissue as heifers grown at slower rates of gain. Heifer calves grown at rates exceeding 0.8 kg/d produced more milk in their first lactation than calves grown for 0.5 kg/d (Bar-Peled et al., 1997; Foldager and Krohn, 1994). Six week old heifers with body weight gains greater than 0.9 kg/d had the same amount of mammary parenchymal tissue as heifers grown for 0.6 kg/d (Sejrsen et al., 1998). However, these studies utilized whole milk in achieving the higher rates of gain. The effects of increasing growth rates using milk replacer on mammary development and future milk production are unknown.

Mammary growth and development

Mammary gland growth occurs in five distinct phases: prenatal, prepubertal, postpubertal, pregnancy and peripartum (Schmidt, 1971). At birth, most of the basic gland structure is developed. Beginning around the third month after birth, the first allometric growth phase is initiated. During the allometric phase, the amount of DNA in the mammary parenchyma increases faster than the body grows (Sinha & Tucker, 1969). In this phase, there is rapid growth of the mammary fat pad, connective tissue, and mammary ducts, but no alveoli are formed (Sejrsen et al., 2000). The parenchymal tissue contains 10-20% epithelial cells, 40-50% connective tissue and 30-40% fat cells. The onset of puberty causes growth of the mammary gland to slow to a rate equivalent to the animal's body growth rate, known as isometric growth. From puberty until conception,

mammary development is limited, but increases during pregnancy. In early pregnancy, extensive lobulo-aveolar development occurs (Sejrsen et al., 2000). Groups of alveoli begin to fill the area previously occupied by fatty tissue in the mammary gland (Tucker, 1969). The parenchyma of the lactating mammary gland consists of 40-50% epithelial cells (ducts and alveoli), 15-20% lumen, approximately 40% connective tissue, and almost no fat cells (Harrison et al., 1983).

Hormonal regulation of mammary development

Mammary glands of prepubertal heifers grow in response to somatotropin and estrogen (Radcliff et al., 2000; Purup et al.,1993). The observed decrease in somatotropin concentrations in heifers on a high feeding level has been implicated in the diet-related inhibition of mammary development. However, the mammary gland does not have somatotropin receptors (Akers, 1985), although it has been shown to have mRNA for the somatotropin receptor (Plath-Gabler et al., 2001). Studies have shown that bovine somatotropin (bST) stimulates mammary growth. Heifers receiving bST had 18% more mammary parenchymal tissue than control heifers (Sejrsen et al., 1986). There was no difference between the two groups in composition of parenchymal tissue. Bovine somatotropin-treated cross-bred beef heifers had an increased amount of mammary parenchymal tissue, less extra-parenchymal fat, and less parenchymal lipid (Carstens et al., 1997). Irrespective of dietary treatments, a high rate of gain (1.2 kg/d) or the control rate of gain (0.8 kg/d), prepubertal heifers injected with

bST had more mammary parenchymal DNA than heifers not receiving bST injections (Radcliff et al.,1997). In another study, Radcliff et al. (2000) found that heifers gaining 1.2 kg/d and receiving bST injections produced a similar amount of milk at an earlier age as heifers gaining 0.77 kg/d and not receiving bST injections.

Somatotropin acts to stimulate growth through increasing insulin-like growth factor-I (IGF-I) synthesis. Animals receiving exogenous bST had higher serum concentrations of IGF-I (Buskirk et al.,1996; Sejrsen et al.,1986). Evidence suggests that bST may act indirectly on the mammary gland through other factors such as IGF-I (Sejrsen et al., 1986). Silva et al. (2002) showed that intramammary infusions of IGF-I increased the number of epithelial cells undergoing DNA synthesis by 60%. Further, Prosser et al. (1990) observed a 25% increase in milk yield from close-arterial infusion of IGF-I in lactating goats.

In vitro, IGF-I stimulates mammary cell proliferation (Purup et al., 1995). In rodents, IGF-I mediates the action of somatotropin in inducing terminal end bud formation (Ruan and Kleinberg, 1999). Ruan and Kleinberg have shown in mice that administration of IGF-I for 5 d to IGF-I $^{(-/-)}$ null mice stimulated mammary development, as indicated by presence of terminal end buds. Administration of IGF-I for 14 d increased terminal end bud numbers significantly and the percent area of the gland occupied by ductal elements was twice that of the 5 d administration. The number of ducts was not significantly increased, but ducts were longer. This accounts for the larger fat pad area occupied by glandular elements. Virgin heifers had higher mRNA expression of IGF-I in mammary

tissue than pregnant heifers or lactating cows (Plath-Gabler et al., 2001). However, IGF-I receptor numbers did not decrease with gestation or lactation. Higher IGF-I expression during mammogenesis might indicate a proliferative role throughout development.

Serum concentrations of IGF-I are increased by feeding high levels of energy (Sejrsen and Purup, 1997). Prepubertal heifers fed for a high rate of gain had increased IGF-I concentrations and decreased somatotropin concentrations, resulting in a decrease in mammary parenchymal growth (Capuco et al.,1995). They suggested and Purup et al. (1996) showed that feeding high amounts of energy reduced sensitivity of mammary tissue to stimulation by IGF-I. In heifers younger than four months of age, feeding for ad libitum intake (0.95 kg/d average daily gain (ADG)) increased serum IGF-I concentrations and decreased somatotropin concentrations (Petitclerc et al., 1999). Feeding for ad libitum intake decreased mammary parenchymal volume by 28% when adjusted for body weight, compared to control heifers gaining 0.62 kg/d.

Leptin is a hormone synthesized by adipocytes in proportion to the amount of lipid stored, and which acts through the central and peripheral nervous systems. It is regulated by a variety of hormones including somatotropin, insulin, and IGF-I (Houseknecht et al., 2000; Smith et al., 2002) and produced by numerous tissues including the mammary gland. Smith et al. (2002) found a decline in leptin production in the presence of high levels of IGF-I. Mammary epithelium has leptin receptors (Smith et al., 2000), which suggests that leptin may regulate mammary growth. Silva et al. (2002) found that leptin decreased

IGF-I induced mammary epithelial cell proliferation.

Effects of feeding level on mammary growth in calves

Sejrsen et al. (1998) proposed that heifer calves can be grown at rapid rates of gain during the first isometric growth phase without impairing future mammary development. When heifers were grown from 5 d of age to 90 kg, over 0.90 kg/d ADG was achieved without inhibiting development of mammary parenchyma tissue (Sejrsen et al., 1998). Heifer calves fed for ad libitum-intake (1.0 kg ADG) until 4 months of age had a volume of mammary parenchyma tissue that was 28% less that that of restricted-fed heifers (Petitclerc et al., 1999). Petitclerc suggested that body weight is not a perfect measure of maturity, but that a more accurate measurement would be bone growth. Petitclerc suggested that late-maturing body parts (fat and reproductive organs) would be more affected by high planes of nutrition than the early maturing parts (bones and brain). Perhaps the calves on the ad libitum-intake diet were in the allometric growth phase. It is during this period when high rates of gain are known to impair mammary development.

In an Israeli study, calves that were allowed to suckle a dam from birth to 6 weeks of age grew faster (0.85 kg/d) than their counterparts (0.56 kg/d) fed milk replacer (MR) at typical amounts (Bar-Peled et al., 1997). The suckled calves were taller than MR-fed calves and producer more milk in their first lactation. In an earlier study by Foldager and Krohn (1994), calves fed whole milk grew faster (1.1 vs. 0.6 kg/d) and produced more milk as cows than restricted-fed calves. These two studies illustrate the potential to grow heifer calves at high

rates of gain without impairing future milk production.

Effects of rapid growth in prepubertal heifers

In the 1950's, Swanson (1960) experimented with different prepubertal growth rates using twin heifer calves. One calf of each twin pair was fed for a high rate of gain while the other was fed for a lower rate of gain. For the first lactation, heifers on the high growth diet produced 15% less milk than their counterparts. Upon slaughter following the second lactation, the cows raised for rapid body weight gains as heifers had less developed mammary parenchymal tissue than heifers raised at slower rates of gain. Swanson also found a difference in parenchymal tissue development between heifers starting the trial at various ages from 4 to 7 months. The mammary glands of heifers starting the trial at a younger age had more extra-parenchymal fat than older heifers. Results from Sejrsen et al. (1982) support Swanson's idea that ad libitum intake by heifers impairs development of mammary secretory tissue. Heifers with feed available ad libitum had more extra-parenchymal fat, 23% less parenchyma and 32% less parenchymal DNA than restricted-fed heifers. The decrease in mammary DNA in prepubertal heifers is a reflection of a reduction in DNA per gram of parenchyma and a decrease in parenchymal mass. There were strong negative correlations between body growth rate in prepubertal heifers and measures of mammary secretory tissue (Sejrsen et al., 1982). This supports the idea that there is a critical period in which growth of secretory tissue is negatively influenced by feeding for increased rates of gain.

Several studies have followed heifers fed for an increased rate of gain in the prepubertal period and measured milk production of control and rapidly-grown heifers. Herman and Ragsdale (1946) observed heifers grown rapidly to have a coarse build and "heavy, meaty udders" prior to freshening. The rapidly-grown heifers produced 120 kg less milk in their first lactation than the normally-grown heifers of the same age. Heifers on an ad libitum intake diet (1.1 kg/d ADG) from 91 kg, the approximate beginning of the first allometric growth phase, until the heifer was confirmed pregnant resulted in lower milk production for multiple lactations (Gardner et al., 1977). The animals that had feed available ad libitum were able to achieve puberty and first calving six months earlier than feed-restricted mates (0.8 kg/d ADG). Heifers on an accelerated growth program were significantly lighter at first calving than the restricted-fed animals, possibly resulting in lower production due to lower body weight. It also concluded that animals reared rapidly continued to have lower milk production and smaller mammary glands that contained less secretory tissue than the heifers on a normal growth diet (Harrison et al., 1983). Histological analysis of the glands showed the mean area of individual alveoli to be similar for both treatments, but the rapidly reared animals had a smaller volume of alveolar tissue.

Little and Kay (1979) suggested that the difference in milk production was due to the rapidly-grown heifers reaching puberty and calving at a younger age. To test their hypothesis, rapidly-grown heifers were bred at the same time that normally grown heifers reached puberty. The rapidly-grown heifers still produced less milk than the normally-grown heifers, but more than rapidly-grown heifers

bred at the onset of puberty. Data suggests that the decrease in milk production could result from a shorter allometric mammary growth period (Van Amburgh et al., 1998; Little and Kay, 1979). It may be that hormones that induce puberty signal an end to the allometric mammary growth.

Effects of rapid growth on postpubertal heifers

Most data have shown negative effects from a high feeding level on mammary growth during the allometric growth phase. During the second isometric growth phase beginning after puberty, the negative effects of increased rate of body weight gain on mammary gland development do not occur (Sejrsen et al., 1982). Postpubertal heifers fed to gain 1.1 kg/d or 0.588 kg/d had a similar amount of parenchymal DNA when expressed per unit of body weight. Lacasse and Block (1993) fed heifers for rapid growth (0.8 kg/d) after puberty and during pregnancy. They observed no difference in milk production or age at first calving in comparison to heifers on moderate growth diets (0.7 kg/d). It might be expected that rapid gain during the second allometric growth phase would decrease milk production. However, Lacasse and Block (1993) suggest that impaired growth did not occur due to the set length of gestation allowing for normal development on either plane of nutrition. During the first allometric growth phase, animals on high planes of nutrition generally have a shorter growing time before they reach puberty and begin the second isometric growth phase.

Effects of rapid growth due to changes in protein and energy

Many studies have demonstrated that a high plane of nutrition impairs mammary development. Most recently, scientists have investigated the importance of both amounts and sources of protein and energy for these rapid growth diets. The effects of corn silage versus alfalfa silage on high growth diets in prepubertal heifers was studied by Capuco et al. (1995). It was found once again that a high plane of nutrition, achieved by increasing the protein intake, increased total mammary gland weight, increased adipose tissue and decreased parenchymal mass. When comparing the two high diets, the high alfalfa silage diet produced the most desirable response in terms of greater parenchymal mass, greater DNA content, and earlier age at puberty. This would indicate that a lower energy and higher protein intake diet would be more beneficial in promoting mammary development.

Heifers fed for high body weight gains (1.0 kg/d) had inhibited mammary development in comparison to heifers grown at slower rates, regardless of protein source (Van Amburgh et al., 1998). On the other hand, Radcliff et al. (1997) reported that heifers fed high protein, high energy diets for rapid rates of gain (1.2 kg/d) did not have impaired mammary development compared with heifers grown at a standard rate. It was concluded that feeding high-energy, high-protein diets to heifers for rapid rates of gain yielded no detrimental effect on milk production (Pirlo et al., 1997). However, the rapid rates of gain for the heifers in the Pirlo et al. study averaged 0.85 kg/d, which is lower than other studies with gains greater than 1.0 kg/d (Radcliff et al., 1997; Van Amburgh et al., 1998). The

heifers consuming the low-protein and low-energy diet and gaining body weight at a slower rate were the same age as the rapidly-grown heifers at puberty and first calving, indicating that the rapidly-grown heifers were not growing at faster rates than the control heifers to decrease the age at puberty. This could be the reason that Pirlo et al. did not see a difference in milk production. Prepubertal heifers fed for rapid growth rates usually reach puberty earlier than normally grown animals because time of puberty is dependent on body weight (Hall et al., 1995). Prepubertal heifers fed for rapid growth (0.9 kg/d ADG) on a diet high in crude protein had a smaller area of fat tissue in the mammary gland and a lower ratio of fat to mammary secretory tissue in comparison to heifers gaining at a normal rate (Dobos et al., 2000). First-lactation milk production was not different between heifers gaining weight at normal and rapid rates. The high crude protein, high rumen-escape protein, high-energy diet decreased the weight of the dry gland and tended to decrease the amount of fat and area of secretory tissue compared to glands from heifers consuming the high-energy, high crude protein, and low rumen-escape protein diet. These studies suggest that protein and energy sources can affect mammary gland growth.

Compensatory growth

Compensatory growth occurs in animals when they have been ill or under fed, and then are re-alimented on a higher nutritional level. Some suggest that dietary energy restriction can increase the life span and productivity of an animal. A number of studies have concluded that growing animals on a stair-step compensatory growth scheme results in faster gains with less feed consumed

and improved feed efficiencies (Park et al., 1987, 1998; Wright and Russel, 1991; Sarkar et al., 1983). During the compensatory growth phase, animals have higher rates of gain compared with controls, which results from a lower basal metabolic level during the lower feeding phase. This could be due to the reduced viscera weight, which allows for a higher proportion of protein and energy being utilized for growth (Hornick et al., 2000). It has also been suggested that leaner animals have lower maintenance levels, which could result in more energy available for growth when they have been restricted fed (Wright and Russel, 1991). There are reports of improved meat quality, but it is unknown whether or not compensatory growth would improve milk production in dairy heifers. Research conducted at North Dakota State University in both rats and dairy heifers demonstrated decreased lipid content of the mammary gland in animals undergoing compensatory growth (Park et al., 1994, 1998). They found that the stair-step growth regime encouraged hyperplasia in mammary tissue, which resulted in an 8-10% increase in milk yield in rats. Beef heifers grown on a stair-step compensatory growth regime showed a 6% increase in milk production, and a tendency towards higher protein and lower fat content of the milk. Due to having a lower maintenance level, restricted-fed animals are able to use more energy for growth during the re-alimentation period. Beef heifers grown on a stair-step regime showed a 2-fold increase in weight gain with a 7% feed efficiency compared to 3.2% for control animals (Park et al., 1998). Park et al., (1994) suggested that restricting energy by 30% results in improved meat and milk quality while not inhibiting growth or reproductive ability.

Following the energy-restricted phase, animals proceed through several phases (Hornick et al., 2000). Compensatory growth initiates at re-feeding and continues for several months until growth rates decline. The period from the beginning of the feeding phase until growth rates peak and then decline is approximately four months. At the initial stages of compensatory growth, muscle and protein are deposited (Wright and Russel, 1991). Depending on the duration of the refeeding state, fat deposition may begin. Frequently adipose tissue develops rapidly and animals that achieve compensatory growth are fatter. The carcass composition can be similar to that of the restriction phase. This also depends on the ability of the animal to deposit lean tissue, which is limited in older animals and dairy breeds (Hornick et al., 2000).

There are two phases of compensatory growth (Wright and Russel, 1991). The initial phase resulted in animals that had higher protein and water content with less fat and energy from the start of the experiment to 350 kg. Then, continued ad libitum feeding from 350 to 400 kg for compensatory growth resulted in animals that were lower in protein and water with higher fat content. These compensatory growth animals were the same body composition as the ad libitum fed animals at 450 kg. This is similar to results that Sarkar et al. (1983) found in growing pigs. By 90 kg body weight, pigs on a compensatory growth regime were the same body composition as the pigs that were on full feed up to 90 kg. Compensatory growth can be beneficial in stimulating lean tissue growth in some animals while in others it promotes additional fat deposition.

Calf Health and Nutrition
Growth rates

Achieving high growth rates in a young calf can be a challenge. Born with a functioning abomasum, the calf develops its digestive system rapidly, with influence from its diet. A calf raised on a liquid diet will have an abnormally developed forestomach, with retarded growth in rumen papillae and thin rumen walls (Davis and Drackley, 1998). While it was once thought that roughage was essential to the development of the rumen and reticulum, it is now known that dry feed with high potential for fermentation is the stimulator of rumen development.

Most farms feed milk replacer to calves at 1.25% of BW on a dry matter basis, but it is possible to feed calves at different levels for different rates of gain. Calves fed milk replacer at 19.5% DM for ad libitum intake, were able to gain 0.87 kg/d with 1.65 kg DM intake using milk replacer (Lineweaver and Hafez, 1969). Calves fed whole milk for ad libitum intake gained 0.962 kg/d ADG compared to 0.686 kg/d and 0.629 kg/d ADG when feeding calves for ad libitum intake on nonfat milk and colostrum, respectively. Huber et al. (1984) fed calves two different quantities of whole milk. One group received a constant amount until weaning and the other group continued to receive increased amounts until d 42 and then milk was decreased until weaning. The calves receiving more milk until d 42 had higher rates of gain than the calves that received a constant amount. The calves receiving more milk until d 42, had lower starter intake than calves receiving a constant amount, but did not differ in total dry matter intake or feed efficiency.

Male Holstein calves were fed milk replacer (30% CP, 20% fat) to achieve

three different body weight gains (treatment 1: 1% BW, 15% DM; treatment 2: 3% BW, 15% DM; treatment 3: 4% BW, 18% DM) (Diaz et al., 2000). By 7 wk of age, calves on treatment 3 had achieved 1.21 kg/d ADG with treatment 1 only gaining 0.52 kg/d ADG. All three treatments were fed only milk replacer and did not have starter available as in other studies (Huber et al., 1984). Calves that were raised on 18% BW milk replacer (24.8% CP, 18.9% fat) at 12% DM gained 1.03 kg/d with no access to calf starter (Bartlett, 2001). Calves fed at 1.25% BW on a dry matter basis only gained 0.36 kg/d, which suggests that this may not provide sufficient energy for optimal growth.

Body composition of calves

Increasing the amount of milk consumed to over 1.75% BW on a dry matter basis increases lean tissue, fat deposition, and the efficiency of gain, but does not affect the composition of gain. Few studies have analyzed the body composition of young calves fed milk replacer (Donnelly and Hutton, 1976ab; Diaz et al., 2000; Bartlett, 2001). One study reported that Holstein bull calves were fed milk replacer for three target rates of gain (0.50, 0.95, 1.4 kg/d) and were slaughtered at three different body weights (65, 85, 105 kg) (Diaz et al., 2001). It was suggested that different rates of gain might affect body composition. The calves fed to gain 1.4 kg/d were unable to achieve the desired target rate of gain, only reaching 1.1 kg/d ADG, while the two lower rates of gain were adjusted weekly to keep the calves from exceeding their target growth rate. The calves on the higher rate of gain were also fed milk replacer reconstituted to 18% DM, while calves on the two lower diets were fed at 15% DM. Calves

targeted for 1.4 kg/d ADG tended to have lower carcass water content, lower CP, and higher fat content. The calves on the 1.4 kg/d ADG diet had a higher feed to gain conversion ratio than the other two treatments. Calves fed for 0.5 kg/d ADG had less fat and more protein deposition than the other two treatments. The calves targeted for 0.95 kg/d and 1.4 kg/d ADG had more fat deposition as the calves increased in body weight, which demonstrated that as rate of gain increased, fat deposition also increased. However, fat deposition and rate of gain did not increase linearly. The heavier calves at slaughter had a higher energy content of tissue gain.

It has been suggested that there may be a desired protein intake concentration to achieve an optimal body composition (Donnelly and Hutton, 1976; Bartlett, 2001; Drackley and Davis, 1998). Donnelly and Hutton (1976) found that increasing the protein concentration in feed from 29.6% to 31.5% resulted in higher carcass fat content and lower protein. Increasing the crude protein from 15.7% to 31.5% showed that there was a point where the body protein increased to its highest percent and fat content decreased to its lowest percentage before reversing the effects. Increasing the energy and protein level of the feed decreased carcass water and fat content and increased the protein percent (Donnelly and Hutton, 1976). Bartlett (2001) suggested that increasing crude protein percentage does in fact lower carcass fat and increase carcass protein, but increasing intake has negative effects. Feeding at a higher percentage of BW from 1.25 to 2.25% on a dry matter basis increased fat content and lowered protein composition. Calves fed for 2.25% BW on a dry matter basis

had 1.03 kg/d ADG while 1.25% BW on a dry matter basis had 0.36 kg/d ADG. One may conclude that calves fed for lower ADG with higher protein diets would have a more optimal body composition.

Much research focuses on understanding how diet affects changes in composition of the mammary gland or body composition. Little research has focused on both. Companion studies reported the effects of diet on mammary gland composition, body composition and milk yield (Waldo et al., 1997 and Capuco et al., 1995). Prepubertal heifers were fed at two different rates of gain with two diets, corn silage and alfalfa silage. During the first lactation treatment did not affect milk production. However, there was a difference in body composition of the heifers. Heifers fed alfalfa silage had less fat, more protein, less water, and more ash than the corn-silage fed animals. The heifers fed for lower ADG had less fat but did not differ in carcass protein, water or ash in comparison to the heifers on higher ADG diets. Heifers on the high corn diet and high alfalfa diet had heavier mammary glands, but had more fat in the total gland. No difference was observed between heifers fed the low-alfalfa and high-alfalfa diets in percent parenchyma and fat of the gland. Compared with heifers fed the low-corn diet, heifers fed the high-corn diet tended to have less mammary parenchyma and a higher fat percentage in the mammary gland. Therefore, feeding a high diet using corn or alfalfa silage negatively affected the mammary gland, while the alfalfa silage diet produced a more desirable carcass in the heifers.

Management and health of calves

Management of the young calf can result in a variety of outcomes. Calves that are housed in individual clean pens, free from drafts and dampness will thrive to be healthy, productive cows. However, heifers that are exposed to disease at a young age may never fully recover from their calfhood ailment and reach their fullest potential. In 1995, the national average for heifer calf mortality rate from birth until weaning was 11.0% (National Animal Health Monitoring System, 1996). From weaning until calving, the mortality rate dropped to 2.4% (National Animal Health Monitoring System, 1996). The high level of mortality in such a short time period from birth until weaning makes this the single most important period of financial risk because of death loss. Total costs per day for heifers during the first three months of life are the greatest of any phase of their growth, due to cost of milk replacers, calf starters, and individual attention from caretakers (Drackley and Davis, 1998). Research has shown that housing can also affect growth rates. Calves raised in hutches outside during the winter as compared with calves in artificially vented barns had better growth rates, higher feed efficiencies, and less medical treatment (Tomkins et al., 1994). Providing proper ventilation for calves housed indoors is a necessity in preventing spread of disease and possible respiratory problems.

Diarrhea is often associated with higher intakes of milk replacer, but there was no difference observed between the treatments in the study by Huber et al. (1984). Researchers attributed no difference in diarrhea to more intense management of the calves and an increase in biosecurity. They found that the

calves receiving more milk to d 42, had higher rectal temperatures than the calves receiving a constant amount, which would indicate more stress or increased metabolic rate associated with receiving more milk. Calves on both diets increased average daily gain as their age increased and peaked at 1.0 kg/d when the calves were 7 wk old.

Heat stress is often discussed when referring to cows, milk production and dry matter intake. Respiratory rates of 20 breaths/min indicate that the animal is near a lower ambient temperature, while 80 breaths/min and possible panting indicate heat stress (Spain and Spiers, 1996). Calves raised in hutches that were provided with shade experienced less heat stress (Spain and Spiers, 1996; Coleman et al., 1996). Increased respiratory rates occur after 26°C in calves (Spain and Spiers, 1996). Calves that were provided shade consumed less feed than calves not provided shade, but had the same ADG resulting in higher feed efficiency (Coleman et al., 1996).

Role of IgG on growth rates

The importance of feeding adequate quality colostrum to newborn calves is often overlooked. It is important for optimal development of passive immunity as well as growth rates in calves. Calves that suckled the dam as compared to being fed 2 L of colostrum had higher serum IgG levels after 24 hours (Quigley et al., 1995). The incidence of scours was lower and calves had a higher feed efficiency. A level of 10 mg/ml IgG has been set as an industry standard that will provide calves with adequate protection (Fowler, 1999). A comparison of over 2000 calves found that calves with serum IgG concentrations above 10 mg/ml,

had a 12% lower mortality rate, fewer total days of scours, a higher rate of gain and better feed conversion (Fowler, 1999). It was also observed that calves with serum IgG concentrations above 10 mg/ml had higher rates of gain and better feed efficiency (Kuhne et al., 2000). At weaning, calves that had lower than 4 mg/ml serum IgG compared to calves above 14 mg/ml had a 28.7 kg difference in body weight (Vann and Baker, 2001). Heifers with higher IgG concentrations had increased rates of gain and decreased mortality rates (Robinson et al., 1998). The time of year was also important in that summer-born calves had higher serum IgG concentrations. They speculate that the reason is that the calves suckled the cow more often in the 24 h period to quench their thirst. It was also found that calves of older and high-producing cows had lower IgG levels at 24 and 48 h resulting from teat leakage prior to calving (DeNise et al., 1989). These same heifer calves were tracked through first lactation. Low IgG calves (>12 mg/ml) had a higher cull percentage for low production as cows. The higher IgG calves produced more milk and fat as cows.

While not all calves may have adequate IgG levels (10 mg/ml), it is possible to supplement or replace IgG with commercial products. Calves fed a commercial colostrum replacer containing 20% IgG, calves were able to achieve 13.6 mg/ml IgG within 24 h after receiving the replacer, but were only able to achieve 8.0 mg/ml from a colostrum supplement (10% IgG) after 24 h (Quigley et al., 2001). Calves fed maternal colostrum (13.78 $\pm$ 0.39 mg/ml) or colostrum supplement (13.96 $\pm$ 0.38 mg/ml) did not differ in mean plasma IgG after 24 h (Mowrey et al., 2001). Health status, scours and grain intake levels were not

different among treatment groups.

MATERIALS AND METHODS

Animals

The experimental protocol was approved by the Michigan State University All University Committee on Animal Use and Care. Female Holstein calves (n=74; 43.8 $\pm$ 3 kg BW) were blocked by date of purchase. Four blocks of calves were purchased primarily from out-of-state sale barns via an agent in either Indiana (Blocks 1 and 2; n =15 and 20 calves, respectively) or from two Michigan dairy farms (Blocks 3 and 4; n = 19 and 20). Criteria for purchasing calves were that they be healthy Holstein heifer calves between 5 and 10 d of age, weighing over 45 kg, and not freemartins. The calves arrived at the Michigan State University Dairy Cattle Teaching and Research Center on February 20, March 20, April 17 and May 29, 2001. Upon arrival, calves were weighed and housed in individual pens or hutches. The calves were bedded on straw, spelt hulls or sawdust depending on availability. Blocks 1 and 2 arrived late in the evening from outside the state. Calves were fed 1.9 L of electrolytes (Pro-Lyte® Plus, Milk Specialties Company, Dundee, Illinois) and 2 to 7 h later were given 1.9 L of milk replacer (Calvita® Supreme, Milk Specialties Company, Dundee, Illinois). Blocks 3 and 4 arrived in mid-afternoon from Michigan farms and were given 1.9 L of milk replacer on their normal afternoon feeding schedule.

The day following arrival, the calves began a week-long adaptation period with feedings of 1.9 L milk replacer (21.3% CP, 21.3% fat on a DM basis

reconstituted to 12.5% DM) at 7 a.m. and 5 p.m. Calves had water available at all times and 100 g/d calf starter (Gold Flake™ Calf Starter, Nutrena Feeds, Winnipeg, Manitoba) beginning on the third day after arrival. Calves were given injections 2 to 72 h after arrival with 3 ml Bo-Se (Schering-Plough Animal Health Corp., Union, NJ), 1 ml Vitamin A&D (Vedco, Inc., St. Joseph, MO), 1 ml TSV-2 per nostril (Pfizer Animal Health, NY, NY), 2 ml Endovac-Bovi (Immvac, Inc., Columbia, MO), 1 ml Nuflor per 13.6 kg BW (Schering-Plough Animal Health Corp.),and 5 ml CDT-Toxoid (Pfizer Animal Health).

Some calves displayed health problems due to pneumonia, salmonella, cryptosporidiosis, mycoplasma ear infections, scours, or heart defects. Six calves from block 1, 10 from block 2, 4 calves from block 3 and 1 calf from block 4 were eliminated from the study as a result of one or more health problems listed. A total of 53 calves remained on the study (9, 10, 15, and 19 in blocks 1 through 4, respectively). Final number of calves in the LL, LH, HL, and HH treatments for each block were 2, 2, 2, 1 for block 1; 2,1, 2, 3 for block 2; 4, 3, 3, 3 for block 3; 3, 4, 3, 4 for block 4, respectively. One calf was later eliminated from the data set after being determined a freemartin.

Feeding and Management of Calves

Following the week-long adaptation period, calves were stratified into highest and lowest body weights and then randomly assigned to 1 of 4 treatments (Table 1). Calves on the low diet (L) received standard milk replacer (Calvita® Supreme, Milk Specialties Company, Dundee, IL; 21.3% CP, 21.3% fat

on a DM basis; Table 2) at 1.25% of BW (10% of BW after reconstituting to 12.5% solids), and were fed starter grain (20.5% CP, 3.4% fat on a DM basis; Gold Flake™ Calf Starter, Nutrena Feeds, Winnipeg, Manitoba) according to the Dairy NRC (2001) to gain 0.40 kg/d average daily gain (ADG) from 2 to 8 wk of age (Table 3; period 1). Calves on the high diet (H) received a high-protein milk replacer (Excelerate™, Milk Specialties Company, Dundee, Illinois; 30.3% CP, 15.9% fat on a DM basis) at 2.25% of BW (15% BW reconstituted to 15% DM) and calf starter (25.0% CP, 3.4% fat on a DM basis ; Herd Builder® Calf Starter, Nutrena Feeds, Winnipeg, Manitoba) for 1.2 kg/d ADG according to the Dairy NRC (2001) from 2 to 8 wk of age. Different composition of diets were used since research has shown that increasing protein intake increases carcass protein percentage and decreases carcass fat percentage. Milk replacer powder was added to a bucket containing 43°C water and mixed thoroughly with a wire whisk. Calves were gradually weaned from milk replacer by 7 wk of age.

From 8 to 14 wk of age (period 2), calves received only calf starter and corn grain. During wk 8, the calves on the wk 8 to 14 L diet received standard calf starter to achieve their target rate of gain (0.4 kg/d) according to the Dairy NRC. The calves on the wk 8 to 14 H diet were fed the high protein calf starter for ad libitum-intake. At 9 wk of age, rolled corn was added to both diets. The new diets contained 70% of the respective calf starter and 30% rolled corn. Final CP percentages were 16.5% for the L diet and 21.3% for the H diet. Initially, the rolled corn was going to be added to the diet at the start of the second period, but due to miscommunications it did not get added until the second wk of the period

for the first group of calves. To keep all groups of calves equal in protocol, we added the corn to the diet during wk 9 for the remaining three groups. Calves had fresh water available at all times.

At each feeding, milk replacer was fed and refusals were weighed and recorded. Calves were visually monitored twice daily for health. If calves were determined to be sick by temperature above 39°C, rapid breathing, or lethargic appearance, and if the calf did not consume any of her milk replacer in 2 consecutive feedings, then calves were fed their milk replacer using an esophageal feeder. Calf starter refusal was also weighed and recorded.

Calves were weighed on 2 consecutive days each week and withers height was measured once per week. The amount of milk replacer offered during the first period on the two diets was adjusted by weekly body weight to maintain desired growth rates for the L and H diets. Calf starter offered was adjusted by weekly body weight for L calves in the second period to achieve the target rate of gain. In the second period, the H calves were given their grain mix free choice. Fecal scores were assessed twice daily for the first 21 d the calves were on the farm (1=dry, hard; 2=soft, formed; 3=pudding-like; 4=mix of liquid and solids; 5=liquid).

Blood Collection and Analysis

Jugular vein blood samples were collected once weekly into 7-ml EDTA Vacutainer® tubes (Becton Dickinson & Co., Rutherford, NJ). Tubes were refrigerated overnight and centrifuged the next day at 1550 × *g* for 20 min.

Plasma was recovered and frozen at -20°C. Three days after calves arrived, 2 jugular vein blood samples were collected from each calf for measurement of IgG concentrations as an indicator of passive immunity and blood was refrigerated overnight. Serum was collected by centrifuging the tubes at 1550 × *g* for 20 min and used in a radial immunodiffusion assay to measure IgG levels (VMRD, Inc., Pullman, WA). Whole blood was used in a quick test kit for IgG levels (Midland BioProducts, Boone, IA).

Hormone Analysis

Plasma samples were assayed for insulin-like growth factor-I (IGF-I) concentrations after removal of binding proteins using acid/ethanol extraction (Bruce et al., 1991). Extracts were used in a radioimmunoassay according to GroPep Pty (Growth Factors and Products and Protocols, Adelaide, Australia). The IGF-I for standards and the primary antibody were from GroPrep and the assay was modified according to Sharma et al. (1994) with Staphylococcus *aureus* protein used in place of the secondary antibody.

Plasma leptin concentrations were determined using a radioimmunoassay by Dr. D. H. Keisler, University of Missouri (Delavaud et al., 2000).

Slaughter Procedure

Calves were slaughtered at 8 and 14 wk of age. There were 5 L diet calves and 6 H diet calves slaughtered at 8 wk of age. The remaining calves were slaughtered at 14 wk of age. The calves slaughtered at 8 wk of age were utilized as two separate treatments independent of the 14 wk old calves.

The day prior to slaughter calves were weighed and jugular vein blood samples were taken. Calves were then fed and allowed 1 h to eat prior to being shipped to the Michigan State University Meats Laboratory at approximately 1630 h. The following morning the calves were slaughtered using captive bolt stunning followed by exsanguination.

Calves were weighed again immediately prior to slaughter, approximately 14 to 16 h after last feeding. Within 30 min of slaughter, the mammary glands were collected. The reproductive tracts were examined to confirm that animals were not freemartins and had not reached puberty. One heifer was determined to be a freemartin and her data was eliminated from the results. A second heifer, in treatment group LL, had a large reproductive tract, but was not a free martin. Her data was not eliminated, but some of her data did raise questions.

Carcass Composition and Analysis

The carcass was split in halves, weighed and chilled for 24 h. The left half carcass was then ground 3 times through a commercial grinder (Autio Company, Astoria, OR) and 2 sub-samples of approximately 150 g were taken after thorough mixing. The samples were frozen at -20°C and later one sample was ground to a powder using liquid nitrogen in a Waring Blender (Waring Products Division, New Hartford, CT). Dry matter was determined by the difference in wet weight after sample was placed in a 105°C oven for 24 h. Ash was determined after 5 h oxidation in a muffle furnace at 500°C. Crude protein was analyzed according to Hach et al. (1997). Fat was determined by Soxhlet ether extraction (AOAC, 1990).

Mammary Tissue Collection

Mammary glands were bisected into right and left hemi-glands. Both halves were weighed. The left half was frozen flat in liquid nitrogen and stored at –20°C. Several samples of parenchyma were collected from the right hemi-gland for later analysis of mRNA expression and histology, neither of which are reported in this thesis. The right rear quarter was sliced open and 2 parenchymal tissue samples were excised. Each sample was weighed and frozen in liquid nitrogen. Samples were stored at –80°C. From the right half, 3 samples were taken for histological analysis. One sample was from the parenchymal tissue closest to the teat canal. Another sample was taken from the parenchymal tissue halfway between the teat canal and the exterior edge of the parenchymal tissue. The last sample was taken on the edge of the parenchymal tissue adjacent to the mammary fat pad. Samples were fixed in 10% neutral buffered formalin solution, and processed through a graded series of ethanol washes.

Mammary Tissue Analysis

The left half of the udder was cut transversely, while frozen, into slices 5 to10 mm thick using a band saw. All slices from both the anterior and posterior ends that did not contain parenchymal tissue were discarded. Slices were then allowed to thaw slightly, and skin, teats, and supramammary lymph nodes were removed. Fat located outside the border of the parenchyma was removed and weighed. This fat was defined as extra-parenchymal fat. The remaining tissue was referred to as parenchymal tissue. Frozen parenchymal

tissue was weighed and ground in a Waring Blender (Waring Products Division, New Hartford, CT) using liquid nitrogen into a fine powder. The powder was mixed and sub-sampled for subsequent analysis of RNA and DNA (Tucker, 1964), dry matter, protein, and fat. Dry matter was determined by the difference in wet weight after the sample was placed in a 105°C oven for 24 h. Ash was determined after 5 h oxidation in a muffle furnace at 500°C. Crude protein was analyzed according to Hach et al. (1997). Fat was determined by Soxhlet ether extraction (AOAC, 1990).

Feed Cost Analysis

Cost of the feeds was calculated using only the amount of milk replacer, calf starter, and corn that the calves consumed. The cost of the milk replacer was $1.65 per kg DM for the standard milk replacer and $1.98 per kg DM for the high protein milk replacer. The standard calf starter was $0.35 per kg DM and the high protein starter was $0.41 per kg DM. The rolled corn was calculated as $0.12 per kg DM.

Statistical Analysis

Data were analyzed using GLM procedure of SAS®. Body growth and mammary data were analyzed in a 2 x 2 factorial arrangement. The main effects in the model were low or high diet from 2 to 8 wk of age (period 1), low or high diet from 8 to 14 wk of age (period 2), block, and interaction of the period 1 diet with period 2 diet. Analysis of IGF-I and leptin also used GLM procedure of SAS® and used heifer within treatment by block as the error term for the main effects of

period 1 diet and period 2 diet. The error term for week of age and the interaction of week of age and the main effects was the residual. Least square means and standard errors were reported. Significance was declared at $P<0.05$, and trends at $P<0.10$.

Table1. Experimental treatments[1].

	LL	LH	HL	HH
Adaptation Period 1 wk	Standard milk replacer fed at 1.9 L per day 100 g/d of standard calf starter			
2 to 7 wk of age	Standard milk replacer at 1.25% BW and standard calf starter fed for BW gain of 0.4 kg/d		High protein milk replacer at 2.25% BW and high protein calf starter fed for BW gain of 1.2 kg/d	
7 to 8 wk of age	Standard calf starter fed for BW gain of 0.4 kg/d		High protein calf starter fed for BW gain of 1.2 kg/d	
8 to 9 wk of age	Standard calf starter fed for BW gain of 0.4 kg/d	High protein calf starter fed for minimum BW gain of 1.2 kg/d	Standard calf starter fed for BW gain of 0.4 kg/d	High protein calf starter fed for minimum BW gain of 1.2 kg/d
9 to 14 wk of age	Standard calf starter and corn fed for BW gain of 0.4 kg/d	High protein calf starter and corn fed for minimum BW gain of 1.2 kg/d	Standard calf starter and corn fed for BW gain of 0.4 kg/d	High protein calf starter and corn fed for minimum BW gain of 1.2 kg/d

[1]Calves fed individually

Table 2. Milk replacer composition on a dry matter basis.

	Low diet[2]	High Diet[3]
Crude Protein,%	21.3	30.3
Crude Fat, %	21.3	15.9
Crude Fiber, %	0.16	0.16
Calcium minimum, %	0.79	0.79
Calcium maximum, %	1.33	1.33
Phosphorus,%	0.74	0.64
Vitamin A, IU/kg	66150	165375
Vitamin D_3, IU/kg	22050	5512.5
Vitamin E, IU/kg	441	110.25

[1]Feed manufacturers guaranteed analysis
[2]Calvita® Supreme, Milk Specialties Company, Dundee, IL
[3]Excelerate™, Milk Specialties Company, Dundee, IL

Table 3. Calf starter and corn composition on a dry matter basis.

	Low diet[1]	High diet[2]	Corn[3]	Low grain diet mix[4]	High grain diet mix[5]
Crude Protein, %	20.5	25.0	7.8	16.5	21.3
Crude Fat, %	3.4	3.4	3.75	3.77	5.3
Crude Fiber, %	7.95	9.0			
Acid Detergent Fiber, %	9.0	12.5			
Calcium, %	0.51	0.51			
Calcium maximum, %	1.02	1.02			
Phosphorus, %	0.51	0.51			
Salt minimum, %	0.40	0.45			
Salt maximum, %	0.57	0.68			
Selenium, ppm	0.3	0.3			
Vitamin A, IU/kg	48510	24916.5			

[1]Gold Flake™ Calf Starter, Nutrena Feeds, Winnipeg, Manitoba (guaranteed analysis)
[2]Herd Builder® Calf Starter, Nutrena Feeds, Winnipeg, Manitoba (guaranteed analysis)
[3]Measured by wet chemistry
[4]70% Gold Flake™ Calf Starter and 30% corn (wet chemistry analysis)
[5]70% Herd Builder® Calf Starter and 30% corn (wet chemistry analysis)

RESULTS

Growth and Body Composition

Initial body weights (BW) were not different at the start of treatments (P=0.46; Table 4). At 8 wk of age, calves on the H diet were 10 kg heavier than the calves on the L diet (P=0.0001). The final body weights for the four treatments were 79.7, 106.3, 87.3, and 120.6 kg for LL,LH, HL, HH, respectively. The diets in both periods significantly affected final body weights, so that at 14 wk of age, LH and HH calves were heavier than LL and HL calves (P=0.0001). Beginning withers heights of calves did not differ (P=0.97). Withers heights tended to increase as a result of the H diet at the end of the first period (P=0.06), and the HH and LH calves increased their withers height in the second period as a result of their diet (P=0.001). There was no interaction of period 1 diet and period 2 diet on withers heights (P=0.81).

The H calves were heavier at the end of the first period as a result of increased rate of body weight gain (P=0.0001; Table 5; Figure 1). The diet in the first period did not affect rate of gain from 8 to 14 wk of age (P=0.99), but the H diet in the second period resulted in calves with higher body weight gains (P<0.0001). There was no interaction of period 1 diet and period 2 diet on body weight gain (P=0.12). At the end of the first period, calves on the H diet had gained more height than the L diet calves (P=0.002). While the diet in the first period did not affect height gain from 8 to 14 wk of age (P=0.29), the calves on

the H diet during the second period gained more height than the L diet calves (P=0.0002). There was no interaction of period 1 diet and period 2 diet for withers height gain (P=0.77). Calves fed the H diet during period 1 tended to have higher weight gain to height gain ratios during period 1 (P=0.08), but lower ratios during period 2 (P=0.09). Calves fed the H diet during period 2 tended to have greater weight gain to height gain ratios during period 2 (P=0.09). There was no interaction of period 1 diet and period 2 diet for weight gain to height gain ratio (P=0.72).

Carcass protein and lipid percentage were not affected by diet in either period (Table 6), but there was an interaction of the two period diets on carcass lipid percentage (P=0.03). Carcass water percentage was similar for the two diets in the first period (P=0.12), but during the second period LL and HL calves had a higher percentage of water (P=0.01). Carcass ash percentage tended to increase in HH and HL calves in the first period (P=0.06); this effect was more apparent during the second period in HH and LH calves(P=0.0001).

Gain to feed ratio was higher in HH and HL calves in the first period (P=0.0001; Table 7) and higher for HH and LH calves in the second period (P=0.01). There was no interaction of the two period diets on gain to feed ratio (P=0.56). There was an interaction of period 1 diet and period 2 diet for daily DMI and for daily energy consumed (P=0.08).

Mammary development

Higher protein and energy intakes in both periods resulted in calves with heavier mammary glands after adjusting for BW (Table 8). The H calves slaughtered at 8 wk of age tended to have more mammary parenchymal tissue per 100 kg BW than L calves (P=0.09). In calves slaughtered at 14 wk of age, mammary parenchymal weight per 100 kg BW was 52% greater for calves fed the high diet, compared to the low diet, during the first period (P=0.02). Period 2 diet did not alter mammary parenchymal weight (P=0.76). Feeding the high diet during either the first or second period increased the mass of mammary extra-parenchymal fat (P<0.03).

Diet during the first period did not alter the percentage of lipid within mammary parenchymal tissue at 14 wk (P=0.53). There was a tendency for calves fed the high diet in the second period to have more parenchymal lipid on a percentage basis (P=0.07). Protein percent within the mammary parenchymal tissue was unaffected by the diet in either period (P>0.56).

The calves on the H diet slaughtered at 8 wk of age had 50% more parenchymal DNA than the L diet calves. This effect, though not significant at 8 wk of age (P=0.3; Table 9), was similar to that in calves slaughtered at 14 wk of age. Mammary parenchymal DNA per 100 kg BW was also 50% higher for HH and HL calves at 14 wk of age than for LL and LH calves (P=0.003). The diet in the second period did not affect the amount of mammary parenchymal DNA at 14 wk of age (P=0.97). Mammary parenchymal RNA per 100 kg BW was higher in the H diet calves slaughtered at 8 wk of age (P=0.02). Mammary parenchymal

RNA per 100 kg BW was also higher in the 14 wk old calves on the high diet during the first period (P=0.009). Diet in the second period did not affect mammary parenchymal RNA (P=0.85). The RNA:DNA tended to be higher in calves fed the low diet in the first period compared to the high diet (P=0.09), but was not altered by diet during the second period (P=0.73).

Feed Cost

Cost of milk replacer was higher for HH and HL calves than for LL and LH calves (P=0.0001, Table 10), but the cost of calf starter during the first period was not different between treatments (P=0.18). In the second period, HH and LH calves had a higher cost of calf starter than LL and HL calves (P=0.001). However, the cost per kilogram of gain in the first period was not different for the two diets (P=0.43). In the second period, the cost per kilogram of gain was not altered by period 2 diet (P=0.82), but was greater for calves fed the high diet in period 1 (P=0.03). There was no interaction of diets on feed cost per kilogram of gain (P=0.54). Overall feed costs were significant for both period diets (P=0.0001) and there was an interaction of the period 1 diet and period 2 diet (P=0.007).

Health

The calves purchased for the first two blocks (from an out-of-state source) had a higher percentage of deaths (P=0.005; Table 11). Calves that passed the commercial immunoglobulin G (IgG) test were more likely to survive than the

calves that failed (P=0.02). Neither body weight upon arrival at the farm nor diet in the first period affected mortality rates of the calves (P=0.13; P=0.39, respectively). Calves with higher fecal scores in the first period were more likely to die (P=0.0006). There was no difference in IgG values due to diets (P=0.52; Table 12). There was no difference in fecal scores among treatments in the first period (P=0.89).

Hormones

The mean insulin-like growth factor-I (IGF-I) concentrations for the four treatments from 2 through 14 wk of age were 83, 103, 109, and 176 ng/ml for LL, LH, HL, and HH, respectively (P=0.0001). Concentrations of IGF-I increased over time (P=0.0001; Figure 2). The HH calves had the highest IGF-I plasma concentration at the end of the trial with LL calves having the lowest concentration. The HL calves had a concentration similar to HH calves during the first period, but then declined to a concentration similar to LL calves in the second period. The LH calves had lower concentrations in the first period and higher concentrations in the second period.

The mean leptin concentrations for the four treatments from 2 through 14 wk of age were 1.2, 1.1, 1.1, and 1.2 ng/ml for LL, LH, HL, and HH, respectively (P=0.53; Figure 3). Mean leptin concentrations were highest at 2 wk of age, then declined at 8 wk of age, then increased at 14 wk of age (1.2, 1.0, 1.1 ng/ml, respectively). The decline at 8 wk of age may have been a result of weaning. In the first period, LL and LH calves tended to have a higher leptin concentration

than HL and HH calves (P=0.06). In the second period, LH and HH calves had higher leptin concentration than LL and HL calves (P=0.004).

Table 4. Body weight and height.

	8 wk of age				14 wk of age							
	L[1]	H[2]	SEM	*P*	L[1]L[1]	L[1]H[2]	H[2]L[1]	H[2]H[2]	SEM	Period 1 diet	Period 2 diet	Interaction
n	5	6			11	10	9	11		*P*		
Body Weight at 2 wk, kg	40.4	44.3	2.8	0.34	43.9	46.1	43.9	43.9	1.5	0.46		
Body Weight at 8 wk, kg	56.8	72.4	4.8	0.05	59.8	61.8	70.3	73.1	2.5	0.0001		
Body Weight at 14 wk, kg					79.7	106.3	87.3	120.6	3.4	0.0016	0.0001	0.30
Body Weight at slaughter, kg	55.2	69.4	4.4	0.05	76.1	101.1	83.8	114.8	3.3	0.002	0.0001	0.33
Withers Height at 2 wk, cm	75.3	78.5	1.7	0.21	77.6	78.2	78.1	77.9	1.0	0.97		
Withers Height at 8 wk, cm	81.6	85.6	1.7	0.13	83.3	84.6	85.1	86.6	1.0	0.06		
Withers Height at 14 wk, cm					88.1	91.8	90.4	94.6	1.2	0.03	0.001	0.81

[1]L = standard milk replacer or standard calf starter
[2]H = high protein milk replacer or high protein calf starter

Table 5. Body weight, withers height gain, and weight gain to height gain ratio.

	8 wk of age				14 wk of age							
	L^1	H^2	SEM	*P*	L^1L^1	L^1H^2	H^2L^1	H^2H^2	SEM	Period 1 diet	Period 2 diet	Interaction
n	5	6			11	10	9	11			*P*	
Body Weight Gain, kg/d (Period 1)	0.39	0.67	0.05	0.008	0.38	0.37	0.63	0.69	0.04	0.0001		
Body Weight Gain, kg/d (Period 2)					0.47	1.06	0.40	1.13	0.05	0.99	0.0001	0.12
Body Weight Gain, kg/d					0.43	0.72	0.52	0.91	0.03	0.0001	0.0001	0.08
Wither height gain, cm (Period 1)	6.4	7.1	0.9	0.59	5.7	6.4	7.0	8.8	0.6	0.002		
Wither Height gain, cm (Period 2)					4.8	7.2	5.3	8.0	0.6	0.29	0.0002	0.77
Wither Height gain, cm					10.5	13.6	12.3	16.7	0.7	0.0007	0.0001	0.33
Weight gain : Height gain, kg/cm (Period 1)	3.1	4.1	0.5	0.18	2.9	2.9	3.8	3.4	0.4	0.08		
Weight gain : Height gain, kg/cm (Period 2)					5.6	9.3	3.3	5.7	1.8	0.09	0.09	0.72
Weight gain : Height gain, kg/cm					3.6	4.5	3.6	4.6	0.2	0.85	0.0001	0.86

[1]L = standard milk replacer or standard calf starter
[2]H = high protein milk replacer or high protein calf starter

Table 6. Carcass weight and composition.

	8 wk of age				14 wk of age							
	L^1	H^2	SEM	*P*	L^1L^1	L^1H^2	H^2L^1	H^2H^2	SEM	Period 1 diet	Period 2 diet	Interaction
n	5	6			11	10	9	11			*P*	
Carcass Weight, kg	29.5	38.0	2.7	0.06	37.8	52.0	41.9	62.1	1.8	0.0003	<.0001	0.10
Carcass weight as a % of live body weight	53.3	54.5	0.6	0.15	49.4	51.4	50.0	54.2	0.7	0.02	0.0001	0.11
Carcass Protein, %[3]	19.8	19.9	0.7	0.92	20.3	19.3	20.4	20.3	0.6	0.33	0.29	0.41
Carcass Lipid, %[4]	4.4	4.3	0.5	0.84	4.4	5.1	5.1	4.4	0.3	0.97	0.90	0.03
Carcass Water, %	71.4	70.9	1.1	0.16	70.1	68.0	68.6	67.5	0.6	0.12	0.01	0.43
Carcass Ash, %	4.4	5.2	0.5	0.15	4.5	7.2	5.8	7.6	0.5	0.06	<.0001	0.32
Carcass Protein, kg[3]	13.8	5.0	7.1	0.39	7.7	10.1	8.6	12.6	0.5	0.0009	<.0001	0.09
Carcass Fat, kg[4]	2.9	1.1	1.5	0.37	1.7	2.7	2.1	2.8	0.2	0.08	<.0001	0.27

[1]L = standard milk replacer or standard calf starter
[2]H = high protein milk replacer or high protein calf starter
[3]Pecent nitrogen x 6.25
[4]Determined by ether extract

Table 7. Feed efficiency ratio, daily dry matter intake and energy consumed per day.

	8 wk of age				14 wk of age							
	L[1]	H[2]	SEM	*P*	L[1]L[1]	L[1]H[2]	H[2]L[1]	H[2]H[2]	SEM	Period 1 diet	Period 2 diet	Interaction
n	5	6			11	10	9	11			*P*	
Gain to Feed, (Period 1)	0.468	0.523	0.02	0.09	0.449	0.412	0.530	0.575	0.02	0.0001		
Gain to Feed, (Period 2)					0.372	0.407	0.316	0.373	0.02	0.02	0.01	0.56
Gain to Feed					0.404	0.410	0.418	0.404	0.01	0.13	0.38	0.69
Daily DMI, kg (Period 1)	0.82	1.3	0.1	0.007	0.8	0.9	1.2	1.2	0.1	0.0001		
Daily DMI, kg (Period 2)					1.3	2.6	1.3	3.0	0.1	0.07	0.0001	0.08
Daily DMI, kg					1.1	1.8	1.2	2.1	0.1	0.0004	0.0001	0.18
Energy Consumed, Mcal/d (Period 1)	3.1	5.1	0.3	0.004	3.2	3.4	4.8	4.9	0.2	0.0001		
Energy Consumed, Mcal/d (Period 2)					4.1	8.4	4.1	9.7	0.4	0.07	0.0001	0.08
Energy Consumed, Mcal/d					3.6	5.9	4.5	7.3	0.3	0.0001	0.0001	0.20

[1]L = standard milk replacer or standard calf starter
[2]H = high protein milk replacer or high protein calf starter

Table 8. Mammary gland weights and composition.

	8 wk of age				14 wk of age							
	L[1]	H[2]	SEM	*P*	L[1]L[1]	L[1]H[2]	H[2]L[1]	H[2]H[2]	SEM	Period 1 diet	Period 2 diet	Interaction
n	5	6			11	10	9	11			*P*	
Total gland weight, g	106	185	16	0.01	210	420	240	630	49	0.01	<.0001	0.07
Total gland weight, g/100 kg body weight	181	255	22	0.04	250	390	270	510	35	0.04	<.0001	0.14
Parenchyma, g	2.1	4.8	1.0	0.08	13	16	22	29	4	0.005	.20	0.48
Parenchyma, g/100 kg body weight	3.1	6.5	1.3	0.09	16	15	24	23	4	0.02	0.76	0.99
Extra-parenchyma, g	10.7	36.0	4.4	0.005	45	110	59	190	18	0.01	<.0001	0.06
Extra-parenchyma, g/100 kg body weight	18.1	49.5	6.1	0.009	52	99	65	153	15	0.03	<.0001	0.17
Parenchymal lipid, g[3,4]					1.7	1.4	2.0	4.5	1	0.06	0.18	0.11
Parenchymal lipid, %[3,4]					8.1	8.9	6.7	12.5	2	0.53	0.07	0.17
Parenchymal protein, g[3,5]					1.4	1.4	2.6	2.9	0.5	0.002	0.73	0.8
Parenchymal protein, %[3,5]					9.9	9.9	9.9	9.7	0.3	0.56	0.63	0.55

[1]L = standard milk replacer or standard calf starter
[2]H = high protein milk replacer or high protein calf starter
[3]Amount of mammary tissue from calves at 8 wk of age was insufficient to conduct analyses
[4]Determined by ether extract
[5]Percent nitrogen x 6.25

Table 9. Mammary parenchymal DNA and RNA.

	8 wk of age				14 wk of age							
	L^1	H^2	SEM	*P*	L^1L^1	L^1H^2	H^2L^1	H^2H^2	SEM	Period 1 diet	Period 2 diet	Interaction
n	2	4			11	10	9	11			*P*	
Parenchymal DNA, mg	9.4	16.7	3.4	0.3	39	45	77	110	16	0.002	0.22	0.41
DNA, mg/100 kg BW	12.2	22.4	0.2	0.3	44	41	85	86	14	0.003	.97	0.90
Concentration DNA, mg/g	1.6	3.0	0.2	0.12	2.55	2.59	3.19	3.52	0.2	0.0007	0.4	0.48
Parenchymal RNA, mg	9	51	3.7	0.07	110	130	190	270	36	0.003	0.10	0.39
RNA, mg/100 kg BW	16.0	68.7	1.7	0.02	130	130	200	220	32	0.009	0.85	0.85
Concentration RNA, mg/g	9.32	9.69	0.05	0.09	8.3	8.2	8.5	9.5	0.4	0.05	0.18	0.13
RNA:DNA[3]					3.4	3.3	2.9	2.8	0.3	0.09	0.73	0.89

[1]L = standard milk replacer or standard calf starter
[2]H = high protein milk replacer or high protein calf starter
[3]Amount of mammary tissue from calves at 8 wk of age was insufficient to conduct analyses

Table 10. Costs for milk replacer and starter.

	8 wk of age				14 wk of age							
	L^1	H^2	SEM	*P*	L^1L^1	L^1H^2	H^2L^1	H^2H^2	SEM	Period 1 diet	Period 2 diet	Interaction
n	5	6			11	10	9	11			*P*	
Milk Replacer, $	27.00	74.60	5.34	0.0005	30.21	31.42	71.46	73.65	2.13	0.0001		
Starter, $ (Period 1)	6.29	6.46	0.69	0.86	5.70	6.39	5.41	5.61	0.41	0.18		
Starter, $ (Period 2)					15.14	35.23	15.17	40.74	1.59	0.08	0.0001	0.08
Total, $	33.30	81.06	5.27	0.0006	51.05	73.04	92.04	120.00	3.47	<0.0001	<0.0001	0.37
Feed Cost/Gain, $/kg gain (Period 1)	2.10	2.94	0.17	0.01	2.41	2.89	3.01	2.73	0.28	0.43		
Feed Cost/Gain, $/kg gain (Period 2)					0.78	0.80	0.92	0.88	0.06	0.03	0.82	0.54
Feed Cost/Gain, $/kg gain					1.59	1.21	2.15	1.59	0.06	0.0001	0.0001	0.007

[1]L = standard milk replacer or standard calf starter
[2]H = high protein milk replacer or high protein calf starter

Table 11. Factors affecting calf mortality[1].

		95% Confidence Interval		
	Odds Ratio	Lower	Upper	*P*
Out of state origin vs. in state	20	2	165	0.005
Failure of quick IgG test vs. pass [2]	4.8	1.3	17.5	0.02
Treatment (Low[3] vs. High[4])	1.8	0.5	5.9	0.39
Fecal Score ($\geq$ 4 vs. < 4)[5]	23	4	138	0.0006

[1]Factors with $P<0.05$ increased calf mortality
[2]Commercal IgG test (<10 mg/ml, failure of passive immunity)
[3]Low diet - milk replacer (21.3%CP, 21.3% fat) for 0.40 kg/d ADG
[4]High diet - milk replacer (30.3%CP, 15.9% fat) for 1.2 kg/d ADG
[5]Fecal (1=dry, 2=soft, 3=pudding-like, 4=mix of liquid and solids, 5=liquid)

Table 12. Fecal score and plasma IgG Values.

	8 wk of age				14 wk of age					
	L^1	H^2	SEM	*P*	L^1L^1	L^1H^2	H^2L^1	H^2H^2	SEM	*P*
n	5	6			11	10	9	11		
Fecal	2.94	3.37	0.15	0.08	2.96	3.04	3.16	3.21	0.12	0.89
IgG, mg/dl	2000	1320	540	0.38	1000	920	1240	940	200	0.52

[1]L = standard milk replacer or standard calf starter
[2]H = high protein milk replacer or high protein calf starter

Figure 1. Daily body weight gains by treatment.

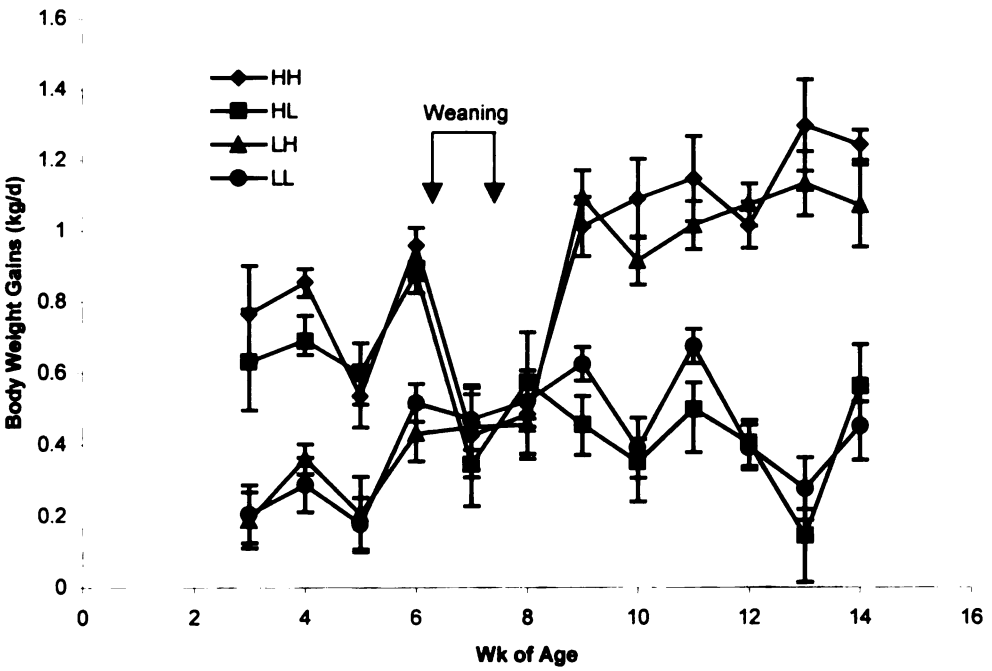


Figure 2. Plasma IGF-I concentrations in heifers for each week of the trial.

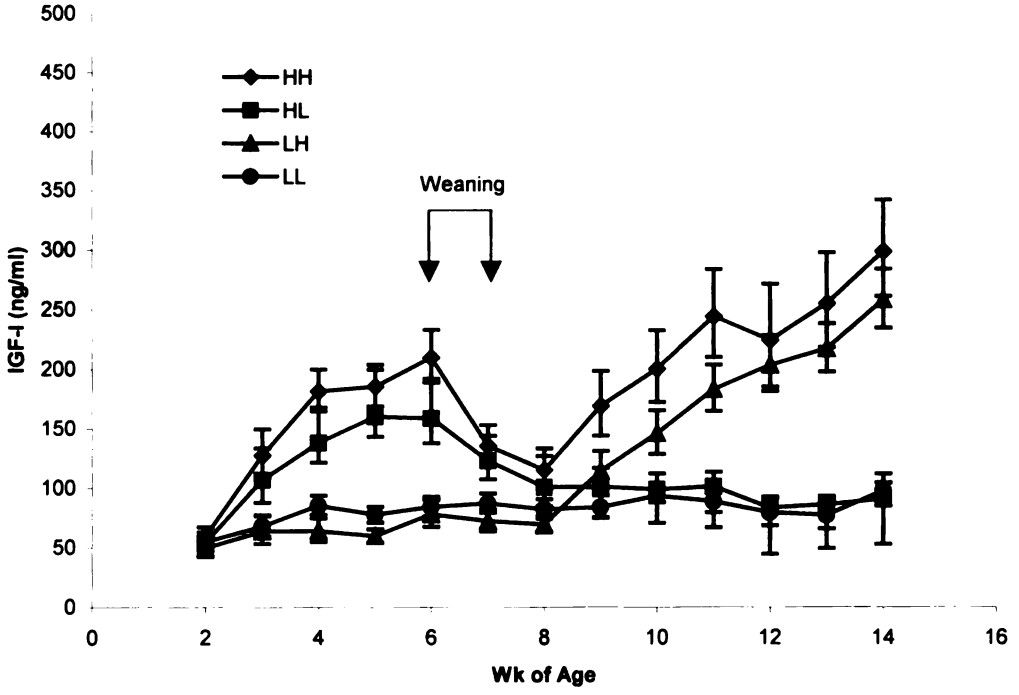
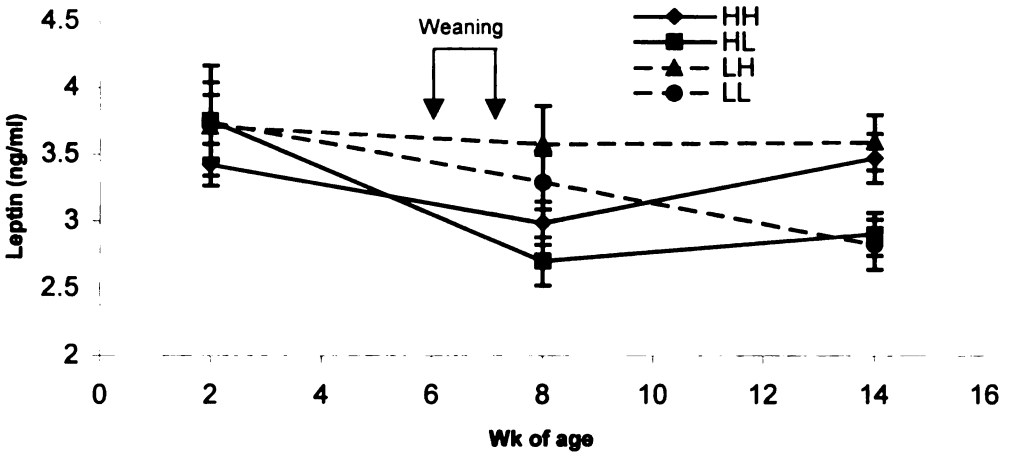


Figure 3. Plasma leptin concentrations for heifers at 2, 8, and 14 weeks of age.



DISCUSSION

This study is the first to examine the effects of increased energy and protein intake, using milk replacer and grain, on mammary development and growth in Holstein heifer calves between 2 and 14 weeks of age. Previous research has shown that increasing energy and protein intake of prepubertal heifers after weaning can inhibit future milk production potential and change carcass composition (Radcliff et al., 2000; Capuco et al., 1995; Waldo et al., 1997). Our results show that increasing energy and protein intake up to a level that promotes 0.66 kg BW gain per day enhances mammary parenchymal growth and increases carcass protein percentage.

Growth

As the H calves consumed additional milk replacer with higher protein and energy intake per day, we expected to see higher rates of gain in turn leading to heavier calves. The calves on the high diet did not achieve their targeted rate of gain in the first period. During the first few weeks of life the digestive tract is rapidly developing, such that energy and protein requirements may vary (Davis and Drackley, 1998). The calves also experienced health problems and transportation stress before their arrival at Michigan State University. There was a 10 kg difference at 8 wk of age, and by the end of the trial there were four distinct mean body weights for the four treatment groups.

The diet in the first period influenced the growth rate of the calves during the second period. The H calves in the first period were larger and had higher maintenance requirements than L calves and consumed more. The LH calves had the potential for compensatory growth. During the second period, LH calves achieved the same growth rate as HH calves with a lower dry matter intake. The LH calves had better feed efficiency during the second period, which is similar to other studies that observed compensatory growth (Kabbali et al., 1992; Abdalla et al., 1988). In some studies, animals in the compensatory growth period have achieved higher rates of gain than full-fed animals on the same diet (Carstens et al., 1991; Abdalla et al., 1998). In our study, we did not see the higher rates of gain in the LH calves. Animals reach their maximum rates of gain (~2 kg/d) for compensatory growth between 30 and 60 days after they begin their increased growth phase (Hornick et al., 2000). The LH calves either achieved their maximum gain for compensatory growth or were not allowed enough time to reach their maximum rate of gain before slaughter.

In treatment groups HL and HH, we observed a depression in growth rates after weaning. Calves on the HH diet did not recover from the weaning stress until 14 to 21 d after weaning, while growth rates in HL calves were affected for the remaining week of the first period. All calves began receiving calf starter after 3 d on the farm to avoid this depression in growth rates after weaning. Milk replacer intake was gradually decreased to try to encourage starter consumption, but desired intakes of calf starter were not achieved. If the calves had transitioned more smoothly, then we may have seen higher rates of gain in

HH calves. At weaning, H calves were only consuming 0.25 kg/d DM of starter. To avoid this decline in dry matter intake after weaning it is recommended, that calves consume at least 0.45 kg/d for 3 consecutive days. However, this only supplies two-thirds of the calf's energy needs for maintenance. To continue to gain weight, the calf should be consuming at least 0.8 to 0.9 kg/d of calf starter (Davis and Drackley, 1998). At 8 wk of age, H and L calves were consuming 1.1 kg/d of starter which resulted in gains of 0.51 kg/d. The HH and LH calves were gaining over 1.0 kg/d at the end of the first week of the second period, but still not at the target rate of gain.

Dairy calves fed milk replacer and concentrate diets are limited in their growth efficiencies (Davis and Drackley, 1998). Unlike other newborn animals, the calf is quickly removed from the dam and fed limited amounts. In contrast, lambs and piglets are allowed to nurse their mothers multiple times during the day, resulting in higher daily intakes and better feed efficiency ratios. In the first period, calves fed the H diet consumed less per kilogram of weight that they gained. In the first 6 wk calves consuming 2.43% of their BW on a dry matter basis gained over 0.90 kg/d and were more efficient in converting feed to gain compared to calves consuming 1.38 to 2.0% of their BW on a dry matter basis (Khouri and Pickering, 1968). The HH calves were more efficient in the second period than LL and HL calves, but likely could have been even more efficient. The H calves may have had less rumen development at weaning because they had consumed more milk replacer than calf starter. Rumen development is stimulated by propionic and butyric acids resulting from starter fermentation

(Davis and Drackley, 1998). The weight gains in HL and HH calves decreased quickly after weaning and HH calves did not reach their targets for another 14 to 21 d, which also explains a decrease in gain to feed ratios. Calves in the LH group were the most efficient in the second period resulting from an initial low plane of nutrition and then a higher plane of nutrition. Animals in compensatory growth generally have better feed conversion rates as seen in LH animals, while animals moving from a high plane to a low plane have poorer efficiency ratios (Abdalla et al., 1988; Park et al., 1987).

Calves were the same height at the start of the trial, but H calves were slightly taller at 8 wk of age. At 14 wk of age, calves in treatment group HH were the tallest while HL and LH were similar. Nutritional restriction did not decrease bone growth (Butler-Hogg, 1984), possibly explaining the similarities in LH and HL. Fifty percent of withers height increase occurs in the first 6 months of life (Kertz et al.,1998). Mature height is determined by genetic potential, but diet and feeding regime can result in animals achieving that genetic potential earlier or being retarded in growth and never achieving their maximum mature size (Owens et al., 1993).

Body weight gain was compared to withers height gain for these calves. This ratio is similar to body mass index which correlates well with relative size of body fat stores in humans (Linder, 1991). For the first and second periods, there was a tendency for the H calves to gain more weight per unit of height that they gained, suggesting more carcass fat. In the second period, LH calves had the highest ratio, suggesting that they had the largest fat reserves and HL calves had

the lowest ratio. Overall, according to the weight to height ratio the H calves stored more energy as fat in the second period; however, carcass fat and protein percentages were similar for all treatments.

The carcass data suggests that the ratio of weight gain to height gain does not correlate well with body fat reserves as it does in humans. There was an interaction of diets for carcass fat percentages, such that calves on the LH and HL treatment had a higher carcass fat percentage. I expected LH calves to be leaner due to compensatory growth and HL calves to have less fat since they lost weight for the first few weeks of the second period. Adult animals or dairy breeds have limited potential for lean mass deposition and fat deposition begins early in the compensatory growth phase (Hornick et al., 2000). Lambs that were restricted in protein intake tended to deposit more fat during the re-feeding phase when protein levels were higher (Drouillard et al., 1991). The severity of feed restriction is greater when imposed on animals at a younger age, with a tendency for less lean tissue growth and more fat accretion during the compensatory growth phase (Carstens et al., 1991). Holstein calves gained less lean and more fat when first fed a high protein diet followed by a low protein diet (Abdalla et al., 1988). Calves on the HL diet also had higher carcass fat than LL calves. During nutritional restriction, water and protein are mobilized earlier and faster than fat in restricted fed sheep (Butler-Hogg, 1984). The rate at which fat, protein and water are mobilized depends on the age of the animal and severity of the restriction. Ash percentage can decline in nutritionally restricted animals, but appears to be more affected in mature animals. Ash percentage was highest in the taller

animals (Butler-Hogg, 1984). Calves on the LH and HH diets had the highest percent ash, but were also the tallest and heaviest calves. Increased bone density and diameter would aid in supporting their larger body weights.

Mammary Development

Heifer calves fed a high-energy diet for ad libitum intakes from 6 wk to 4 mo of age had greater mammary volume (335 cm^3) than control heifers (1067 cm^3) (Petitclerc et al., 1999). Daily gains for the ad libitum heifers were 0.95 kg/d and 0.62 kg/d for the control heifers. Petitclerc et al. also found that adjusting the mammary volume for body weight resulted in the ad libitum calves having less volume than the control animals. In my study, after adjusting the gland weight for BW of calves, the calves on diet H diet in both periods had larger mammary glands. However, measurement of mammary volume alone can be misleading because extra-parenchymal tissue is not distinguished from parenchymal tissue.

Calves fed the high diet in the first period had a larger mammary parenchymal tissue mass per 100 kg of BW compared to calves on the low diet. Calves on a high feeding level had a similar mass of mammary parenchyma compared with calves on a moderate feeding level in a study by Sejrsen et al. (1998). Petitclerc et al. (1999) found an increase in parenchymal volume in ad libitum fed heifers, but there was 28% less parenchymal volume after adjusting for body weight. In my study, calves were fed the high and low diets beginning at 2 wk of age. The reason that Petitclerc et al.(1999) noted different effects of treatments may be that their heifers did not start treatments until 6 weeks of age.

Heifers fed from 13 weeks of age (118 kg BW) to 200 kg BW on a high plane of nutrition (1.4 kg/d ADG) and then a low plane (0.88 kg/d ADG) had similar amounts of mammary parenchymal DNA as heifers fed a low plane of nutrition for both periods (Niezen et al., 1996). One explanation is that calves on the high plane of nutrition were 5 months old when they switched to the low plane of nutrition. It may be possible to increase growth rates in the first weeks of the allometric growth phase and not impair mammary growth in prepubertal heifers. Another explanation for not seeing a difference in mammary development for the two treatments was that both impaired mammary growth to the same extent. It was not evident since there was not a lower rate of gain group for comparison.

Mammary parenchymal lipid percentage was increased from the diet in the second period for H calves, probably resulting from increased energy intake. There was no difference in mammary parenchymal protein percentage. Prepubertal heifers on a high corn diet gaining 1.0 kg/d were found to have a higher parenchymal lipid percentage than heifers growing at 0.78 kg/d (Capuco et al., 1995). The high corn diet heifers had the same amount of mammary parenchymal tissue, but there was an increase in adipocytes and a decrease in epithelial cells. The primary ducts were elongated to the same extent in all treatments, but there was less branching of the ducts in the low corn diet animals. This suggests that mammary adipose tissue might present a barrier for epithelial cell proliferation. During the first period, there was no difference in parenchymal lipid percentage and this is the period that parenchyma and parenchymal DNA were increased. In the second period, the calves on the H diet

tended to increase mammary parenchymal lipid percentage and there was no increase in parenchymal growth. This also suggests that adipocytes might serve as a barrier.

Accompanying the increase in parenchymal mass in the first period of the H calves, there was also an increase in parenchymal DNA and RNA concentration. The amount of DNA is an indicator of the number of secretory cell numbers and RNA is an indicator of metabolic activity (Tucker, 1969). The RNA:DNA ratio was measured as an indicator of synthesis activity per cell (Tucker, 1969). The increase in DNA suggests that HH and HL calves would produce more milk as cows. Heifers with similar body weight gains as H calves in my study produced more milk as cows (Bar-Peled et al., 1997). In a Danish study, Foldager and Krohn (1994) determined that heifers with higher weight gains (1.1 kg/d) produced more milk as cows than heifers fed for 0.58 kg/d. However, in both of these studies high growth rates were achieved by feeding whole milk. It is possible that there are other factors, such as hormones and growth factors, in cow's milk that influence mammary development and milk production that would not be found in commercial milk replacers.

Feed Cost

The increased total feed cost of the high diet was expected because calves were consuming more grain and milk replacer. Furthermore, their feedstuffs were higher in protein, making them more costly. Kertz et al. (1998) observed that cost per kilogram of body weight gain and cost per centimeter of

height gain was least in the first six months of life. The costs that we calculated may not be a fair comparison of the cost of each treatment, because we did not include costs related to medical expenses, labor, or costs of refused feed. Calves fed the high diet in period 1 did not have more health problems, but they did require more labor to feed (unrecorded observation) and had more refused feed (data not shown). Moreover, even though feeding the high diet during period 1 was more costly, this diet increased body weight and mammary development so it has the potential to promote puberty at a younger age and increase subsequent milk production. Therefore, the high diet in period 1 might increase profit despite the higher milk replacer cost. If similar increases in growth and mammary development could be achieved with cheaper milk replacer fed at a higher rate or with increased grain intake, the economics would likely favor calf growth rates similar to our high period 1 calves.

Health

Many producers believe that increasing milk replacer intake also increases the incidence of diarrhea in calves. There was no difference in fecal scores between the treatments. No difference was observed in fecal scores between calves on a low and high intake milk replacer, but there was an increase in rectal temperatures suggesting more stress to calves consuming more milk replacer (Huber et al., 1984). Calves on a lower intake milk replacer diet had higher mortality rates than calves on a high intake diet (Williams et al., 1981). Davis and Drackley (1998) found no difference in mortality rates between calves fed for

standard and rapid growth but reported that calves consuming more were healthier and more vigorous.

In this study, calves were purchased from two agents who collected the calves from either sale barns or local farms. The calves purchased in the two first blocks were purchased from sale barns in New York and Pennsylvania and then shipped to Michigan were found to be more likely to die due to transportation stress. The calves purchased for the last two blocks were transported 2 h from two Michigan dairy farms to the Michigan State University Dairy Farm.

Calves with low serum IgG concentrations were more likely to die. These calves had failure of passive immunity making them more susceptible to illness. Also calves having higher fecal scores had a higher mortality rate. If a calf is scouring, then probably there is a pathogen causing the scouring. The body weight of the calves and treatment did not affect mortality rates. This could be expected since illness is caused by pathogens. Calves with failure of passive immunity are going to become ill not due to weight, but due to immune status.

Hormones

At 2 wk of age, insulin-like growth factor-I concentrations in calves in our study were 50 ng/ml and then began to increase. In another study, calves at 2 wk of age had serum IGF-I concentrations of 40 ng/ml (Breier et al., 1988). There was a positive correlation between birth weights and IGF-I concentration. Positive correlations have also been found between average daily gain, body weight and serum IGF-I concentrations (Kerr et al., 1991; Petitclerc et al., 1999;

Nosbush et al., 1996). Male calves at three different body weights and the same average daily gains had increasing IGF-I concentrations as body weight increased (Smith et al., 1998). They also showed that as body weights remained the same, but average daily gain increased there was an increase in IGF-I concentrations. Petitclerc et al. (1999) found that heifer calves on ad libitum intake had higher concentrations of IGF-I. At weaning, IGF-I concentrations in ad libitum intake animals dropped by 100 ng/ml, similar to our results. Prepubertal heifers fasted for two days dropped 200 ng IGF-I per ml, but changes in growth hormone concentrations were not observed (Amstalden et al., 2000).

Heifers consuming a more concentrated diet with more energy had increased serum IGF-I concentrations (Nosbush et al., 1996), which agrees with the results from Capuco et al. (1995) and our study. In the study by Capuco et al. (1995), prepubertal heifers on high energy diets were found to have higher IGF-I concentrations than animals on lower energy diets. The heifers fed for rapid gain had a similar amount of mammary parenchymal tissue to animals gaining more slowly, but were found to have more mammary fat.

IGF-I is a potent mitogen for mammary epithelial cells. Intramammary infusions of IGF-I increased the number of epithelial cells undergoing DNA synthesis in prepubertal heifers (Silva et al., 2002). Infusion of IGF-I into the gland of pregnant beef heifers resulted in an increase in total gland weight and the amount of DNA per gland (Collier et al., 1993). Therefore, the higher serum IGF-I concentrations in the first period for H heifers in my study might have been partly responsible for the increased mammary parenchymal DNA.

Leptin concentrations have been highly correlated with body fat percentage in cattle (Ehrhardt et al., 2000). Sansinanea et al. (2001) found that heifers gaining 0.27 kg/d had lower serum leptin concentrations than heifers gaining 0.80 kg/d. However, in my study the calves on the H and L diet did not differ in carcass fat percentage, but the H calves had lower serum leptin concentrations at 8 wk of age. During the first period, there was a tendency for more total carcass fat in the H diet calves. The H calves had more total carcass fat during the second period possibly explaining the higher leptin concentrations at 14 wk of age.

There was a tendency for a higher percentage of fat in the mammary parenchymal tissue in the calves on the H diet in the second period, HH and LH. These calves also had higher IGF-I and leptin levels at 14 wk of age. Insulin-like growth factor-I has been shown to be a stimulator of mammary development; however, we did not see an effect of diet on mammary development during the second period. Because leptin is secreted by adipocytes, the adipocytes in the parenchymal tissue may have been secreting leptin, which inhibits IGF-I induced proliferation of mammary epithelial cells (Silva et al., 2002). This could explain why we observed no increase in parenchymal tissue mass or parenchymal DNA in the second period in calves on the H diet. Prepubertal heifers receiving intra-mammary infusions of leptin had less epithelial cell proliferation (Silva et al., 2002).

Mounzih et al. (1998) and Malik et al. (2001) observed that ob/ob mice, which do not produce leptin, did not lactate upon parturition. In two trials, ob/ob

mice were injected with leptin to allow the mice to become pregnant. Ob/Ob mice receiving leptin injections through gestation and parturition lactated and raised their offspring. Ob/Ob mice that did not receive continuous injections of leptin failed to lactate. These researchers examined the mammary glands of the mice that did not lactate and found the mammary glands were not fully developed. These two studies suggest that leptin plays a role in mammary development, but its function is unclear.

Implications

From the early 1900's, researchers found that increasing rates of gain in prepubertal heifers resulted in decreased future milk production (Herman and Ragsdale, 1946). Few studies have focused on increasing rates of gain in younger heifer calves. Bar-Peled et al. (1997) and Foldager and Krohn (1994) found that heifer calves consuming cow's milk gained over 0.8 kg/d and produced more milk as cows than calves fed whole milk or milk replacer for body weight gains of 0.6 kg/d.

In this study, we found that heifer calves, between 2 and 8 wk of age, raised on higher energy and protein intake had more mammary parenchyma, more parenchymal DNA, and more extra-parenchymal tissue. During the second period, 8 to 14 wk of age, extra-parenchymal tissue and parenchymal lipid percentage increased. I found no difference in carcass protein percentage between the diets during the two periods. I suggest that increased energy and

protein intake in heifer calves before 8 wk of age can lead to additional milk production once they become cows.

SUMMARY AND CONCLUSIONS

Increasing energy and protein intake from 2 to 8 wk of age increased parenchymal mass, DNA, and RNA concentration in mammary glands of Holstein heifer calves. The increase in energy and protein intake from 8 to 14 wk of age did not stimulate any additional growth of mammary parenchymal tissue or DNA concentration, but continued to stimulate extra-parenchymal fat deposition.

Increasing energy and protein intake from 2 to 8 wk of age increased weight and height of calves and gain to feed ratios, but did not alter carcass protein percentage. Increasing energy and protein intake from 8 to 14 wk of age increased height and weight of calves and gain to feed ratios, but decreased carcass water and ash. Calves on the low energy and protein diet weighed less and were shorter. They were also less efficient at converting feed to gain in both periods. In addition, the calves fed the low diet for both periods had similar carcass protein content and more carcass water than calves on the high diet for either period. The higher energy and protein intake in heifer calves increased plasma IGF-I concentrations.

I conclude that increasing protein and energy intake of 2 to 8 wk old Holstein heifer calves to promote body weight gains of 0.66 kg/d increases mammary development. Whether the increased cost associated with this faster growth is economically beneficial in the long term (through changes in subsequent growth, reproduction, and milk production) must be determined.

FUTURE RESEARCH

The results of this project have stimulated many ideas for future projects. One potential project is to repeat the study and follow the heifers through first lactation to see if the increased mammary development will result in increased milk production. It would be beneficial to slaughter heifers throughout the trial to measure mammary development. A second project might include feeding heifers on the low and high diet from birth until weaning at 7 wk of age. From weaning until first lactation, the heifers should be managed the way that the farm would normally manage their animals.

A third study, similar to the study of Sinha and Tucker (1969), would be useful in determining the points at which isometric and allometric growth begin. From the results of their study, we have estimates of these ages with one rate of gain. Results from this study show that allometric growth can change by increasing rates of gain. Also, the question arises if there is even a first isometric phase or do animals begin the allometric phase at birth.

A final study stems from the idea that cow's milk is better than feeding milk replacer to young calves. In the study by Bar-Peled et al. (1997) and Foldager and Krohn (1994), the heifer calves that suckled dams produced more milk as cows than the calves fed whole milk or milk replacer at restricted amounts. The rates of gain in the animals were different. Calves could be fed milk replacer or whole milk for the same body weight gains until weaning and then managed the

animals the same until first lactation. Milk production during the first lactation would determine if other factors in cow's milk stimulate additional mammary development.

Results from this project have definitely stimulated some interesting thoughts and ideas. It could also result in producers managing their heifer calves differently from birth to weaning.

APPENDIX

Lipid extraction from DNA-RNA assay

During the DNA-RNA assay, 5 extractions occurred in which solution was collected. This solution contained lipid extracts from the mammary parenchymal tissue. The 5 extracted solutions were combined in 1 tube, and allowed to evaporate at room temperature. The remaining solvent was subsequently evaporated in a heated sand bath with forced nitrogen gas, leaving a lipid residue in the bottom of the 25 ml screw-top tube. The lipid residue was re-suspended in 10 ml of a solution of 3 parts hexane and 2 parts isopropanol. The tubes were capped and vortexed for 15 s. After 5 min, the tubes were vortexed again for 5 s. At least 5 min after the last vortex, 5 ml of 6.7% Na sulfate was added to the tubes, which were capped and vortexed once again for 15 s. Then the organic and aqueous phases were allowed to separate. Using a pipette, the supernatant was transferred into labeled and tarred 16 x 25 mm disposable test tubes. The interface of the organic and aqueous solution was washed twice with a 2 ml solution of 7 parts hexane and 2 parts isopropanol. After each wash, the phases were allowed to separate before the supernatant was transferred into the appropriate tube. The solvent was then evaporated in the heated sand bath with forced nitrogen gas and the tarred tube was weighed. Results from the Soxlet extraction and this method were compared. It was found that this method did not give accurate results and data were not reported.

LITERATURE CITED

LITERATURE CITED

Abdalla, H.O., D.G. Fox, and M.I. Thonney. 1988. Compensatory gain by Holstein calves after underfeeding protein. J. Dairy Sci. 66:2687-2695.

Akers, R. M. 1985. Lactogenic hormones: binding sites, mammary growth, secretory cell differentiation and milk biosynthesis in ruminants. J. Dairy Sci. 68:501-519.

Akers, R.M. 1990. Lactation physiology: a ruminant animal perspective. Protoplasma 159:96-111.

Amstalden, M., M.R. Garcia, S.W. Williams, R.L. Stanko, S.E. Nizielski, C.D. Morrison, D.H. Keisler, and G.L. Williams. 2000. Leptin gene expression, circulating leptin, and luteinizing hormone pulsatility are acutely responsive to short-term fasting in prepubertal heifers: relationships to circulating insulin and insulin-like growth factor-I. Biol. Reprod. 63:127-133.

Association of Official Analytical Chemists International. 1990. Official methods of analysis. Vol. I. 15th ed. AOAC, Arlington, VA.

Bar-Peled, U., B. Robinzon, E. Maltz, H. Tagari, Y. Folman, I. Bruckental, H. Voet, H. Gacitua, and A.R. Lehrer. 1997. Increased weight gain and effects on production parameters of Holstein heifer calves that were allowed to suckle from birth to six weeks of age. J. Dairy Sci. 80:2523-2528.

Bartlett, K.S. 2001. Interactions of protein and energy supply from milk replacers on growth and body composition of dairy calves. MS Thesis, University of Illinois, Urbana-Champaign, Illinois.

Breier, B.H., P.D. Gluckman, and J.J. Bass. Plasma concentrations of insulin-like growth factor-I and insulin in the infant calf: ontogeny and influence of altered nutrition. J. Endocrinol. 119:43-50.

Bruce, L.A., T. Atkinson, J.S.M. Hutchinson, R.A. Shakespear, and J.C. MacRae. 1991. The measurement of insulin-like growth factor I in sheep plasma. J. Endocrinol. 128:R1.

Buskirk, D.D., D.B. Faulkner, W.L. Hurley, D.J. Kesler, F.A. Ireland, T.G. Nash, J.C. Castree, and J.L. Vicini. 1996. Growth, reproductive performance, mammary development, and milk production of beef heifers as infuenced by prepubertal dietary energy and administration of bovine somatotropin. J. Anim. Sci. 74:2649-2662.

Butler-Hogg, B.W. 1984. Growth patterns in sheep: changes in the chemical composition of the empty body and its constituent parts during weight loss and compensatory growth. J. Agric. Sci. 103:17-24.

Capuco, A. V., J.J Smith, D.R. Waldo, and C.E. Rexroad, Jr. 1995. Influence of prepubertal dietary regimen on mammary growth of Holstein heifers. J. Dairy Sci. 78:2709-2725.

Carstens, G. E., D.E. Glaser, F.M. Byers, L.W. Greene, and D. K. Lunt. 1997. Effects of bovine somatotropin treatment and intermittent growth pattern on mammary gland development in heifers. J. Anim. Sci. 75:2378-2388.

Carstens, G.E., D.E. Johnson, M.A. Ellenberger, and J.D. Tatum. 1991. Physical and chemical components of the empty body during compensatory growth in beef steers. J. Anim. Sci. 69:3251-3264.

Coleman, D.A., B.R. Moss, and T.A. McCaskey. 1996. Suppl.al shade for dairy calves reared in commercial calf hutches in a southern climate. J. Dairy Sci. 79:2038-2043.

Collier, R.J., M.F. McGrath, J.C. Byatt, and L.L. Zurfluh. 1993. Regulation of bovine mammary growth hormones: involvement of receptors, growth factors and binding proteins. Livest. Prod. Sci. 35:21-33.

Davis, C.L., and J.K. Drackley. 1998. The Development, Nutrition, and Management of the Young Calf. Iowa State University Press, Ames, IA.

Delavaud, C., F. Bocquier, Y. Chilliard, D.H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165:519-526.

DeNise, S.K., J.D. Robinson, G.H. Stott, and D.V. Armstrong. 1989. Effects of passive immunity on subsequent production in dairy heifers. J. Dairy Sci. 72:552-552.

Diaz, M.C., M.E. Van Amburg, J.M. Smith, J.M. Kelsey, and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. J. Dairy Sci. 84:830-842.

Dobos, R.C. K.S. Nandra, K. Riley, W. J. Fulkerson, I.J. Lean, and R.C. Kellaway. 2000. The effect of dietary protein level during the pre-pubertal period of growth on mammary gland development and subsequent milk production in Friesian heifers. Livest. Prod. Sci. 63:235-243.

Donnelly, P.E., and J. B. Hutton. 1976a. Effects of dietary protein and energy on the growth of Friesian bull calves. I. Food Intake, growth, and protein requirements. N.Z.J. Agric. Res. 19:289-297.

Donnelly, P.E., and J. B. Hutton. 1976b. Effects of dietary protein and energy on the growth of Friesian bull calves. II. Effects of level of feed intake and dietary protein content on body composition. N.Z.J. Agric. Res. 19:409-414.

Drouillard, J.S., T.J. Klopfenstein, R.A. Britton, M.L. Bauer, S.M. Gramlich, T.J. Wester, and C.L. Ferrell. 1991. Growth, body composition, and visceral organ mass and metabolism in lambs during and after metabolizable protein or net energy restrictions. J. Anim. Sci. 69:3357-3375.

Ehrhardt, R.A., R.M. Slepetis, J. Siegal-Willot, M.E. Van Amburgh, A.W. Bell, and Y.R. Boisclair. 2000. Development of a specific radioimmunoassay to measure physiological changes of circulating leptin in cattle and sheep. J. Endocrin. 166:519-528.

Foldager, J. and C.C. Krohn. 1994. Heifer calves reared on very high or normal levels of whole milk from birth to six to eight weeks of age and their subsequent milk production. Proc. Soc. Nutr. Physiol. 3:301. (abstr).

Fowler, M. 1999. What is it worth to know a calf's Ig level? Pages 31-36 *in* Proc. 3rd Professional Dairy Heifer Growers Assoc., Bloomington, MN.

Gardner, R. W., J.D. Schuh, and L.G. Vargus. 1977. Accelerated growth and early breeding of Holstein heifers. J. Dairy Sci. 60:1941-1948.

Hach, C.C., B.K. Bowden, A.B. Lopelove, and S.V. Brayton. 1987. More powerful peroxide Kjeldahl digestion method. JAOAC 70(5):783.

Hall, J.B., R.B. Staigmiller, R.A. Bellows, R.E. Short, W.M. Moseley, S.E. Bellows. 1995. Body composition and metabolic profiles associated with puberty in beef heifers. J.Anim. Sci. 73:3409-3420.

Harrison, R.D., J.P. Reynolds, W. Little. 1983. A quantitative analysis of mammary glands of dairy heifers reared at different rates of live weight gain. J. Dairy Res. 50:405.

Heinrichs, A.J. 1993. Raising dairy replacements to meet the needs of the 21st century. J. Dairy Sci. 76:3179.

Herman, H.A., and A.C. Ragsdale. 1946. The influence of quantity and quality of nutrients on the growth of dairy heifers. J. Anim. Sci. 5398. (abstr).

Hornick, J.L., C. Van Eenaeme, O. Gerard, I. Dufrasne, and L. Istasse. 2000. Mechanisms of reduced and compensatory growth. Dom. Anim. Endocrinol. 19:121-132.

Houseknecht, K.L., C.P. Portocarrero, S. Ji, R. Lemenager, M.E. Spurlock. 2000. Growth hormone regulates leptin gene expression in bovine adipose tissue: correlation with adipose IGF-I expression. J. Endocrinol. 164;51-57.

Huber, J.T., A.G. Silva, O.F. Campos, and C.M. Mathieu. 1984. Influence of feeding different amounts of milk on performance, health, and absorption capability of baby calves. J. Dairy Sci. 67:2957-2963.

Kabbali, A., W.L. Johnson, D.W. Johnson, R.D. Goodrich, and C.E. Allen. 1992. Effects of compensatory growth on some body component weights and on carcass and noncarcass composition of growing lambs. J. Anim. Sci. 70:2852-2858.

Kerr, D.E., B. Laarveld, M.I. Fehr, and J.G. Manns. 1991. Profiles of serum IGF-I in calves from birth to eighteen months of age and in cows throughout the lactation cycle. Can. J. Anim. Sci. 71:695-705.

Kertz, A.F., B.A. Barton, and L.F. Reutzel. 1998. Relative efficiencies of wither height and body weight increase from birth until first calving in Holstein cattle. J. Dairy Sci. 81:1479-1482.

Khouri, R.H., and F.S. Pickering. 1968. I. Performance of calves fed on different levels of whole milk relative to body weight. N.Z. J. Agric. Res. 11:227-236.

Kuhne, S., H.M. Hammon, R. M. Bruckmaier, C. Morel, Y. Zbinden, and J.W. Blum. 2000. Growth performance, metabolic and endocrine traits, and absorptive capacity in neonatal calves fed either colostrums or milk replacer at two levels. J. Anim. Sci. 78:609-620.

Lacasse, P., and E. Block. 1993. Effect of plane of nutrition of dairy heifers before and during gestation on milk production, reproduction, and health. J. Dairy Sci. 76:3420-3427.

Lammers, B.P., A.J. Heinrichs, and R.S. Kensinger. 1999. The effects of accelerated growth rates and estrogen implants in prepubertal Holstein heifers on estimates of mammary development and subsequent reproduction and milk production. J. Dairy Sci. 82:1753-1764.

Larson, B.L. ed. 1985. Lactation. The Iowa State University Press, Ames, IA.

Linder, M.C. ed. 1991. Nutritional Biochemistry and Metabolism. Appleton and Lange, Norwalk, Connecticut.

Lineweaver, J.A., and E.S.E. Hafez. 1969. Feed intake and performance in calves fed ad libitum and four times daily. J. Dairy Sci. 52:2001-2006.

Little, W., and R.M. Kay. 1979. The effects of rapid rearing and early calving on the subsequent performance of dairy heifers. Anim. Prod. 29:131-142.

Malik, N.M., N.D. Carter, J.F. Murray, R.J. Scaramuzzi, C.A. Wilson, and M.J. Stock. 2001. Leptin requirement for conception, implantation, and gestation in the mouse. Endocrinol. 142:5198-5202.

Marshall, S.P., and K.L. Smith. 1965. Effect of different milks and levels of intake upon growth of young Dairy calves. J. Dairy Sci. 53:1622-1626.

Mounzih, K., J. Qiu, A. Ewart-Toland, and F.F. Chehab. 1998. Leptin is not necessary for gestation and parturition but regulates maternal nutrition via a leptin resistance state. Endocrinol. 139:5259-5262.

Mowrey, C.M., R.E. James, J.D. Quigley, and M.L. McGilliard. 2001. Absorption of immunoglobulin G in calves fed colostrum or colostrum replacement and animal plasma in milk replacer. J. Dairy Sci. 84(Suppl. 1): 266.

National Animal Health Monitoring System. 1996. Part II Changes in the U.S. Dairy Industry: 1991-1996. Ft. Collins, CO:USDA:APHIS:VS.

National Research Council. 2001. Pages 214-233 *in* Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Niezen, J.H., D.G. Grieve, B.W. McBride, and J.H. Burton. 1996. Effect of plane of nutrition before and after 200 kilograms of body weight on mammary development of prepubertal Holstein heifers. 1996. J. Dairy Sci. 79:1255-1260.

Nosbush, B.B., J.G. Linn, W.A. Eisenbeisz, J.E. Wheaton, and M.E. White. 1996. Effect of concentrate source and amount in diets on plasma hormone concentrations of prepubertal heifers. J. Dairy Sci. 79:1400-1409.

Owens, F.N., P. Dubeski, and C.F. Hanson. 1993. Factors that alter the growth and development of ruminants. J. Anim. Sci. 71:3138-3150.

Park, C.S., M.G. Baik, W.L. Keller, and W.D. Slanger. 1994. Dietary energy restriction-mediated growth and mammary development in rats. J. Anim. Sci. 72:2319-2324.

Park, C.S., R.B. Danielson, B.S. Kreft, S.H. Kim, Y.S. Moon, and W.L. Keller. 1998. Nutritionally directed compensatory growth and effects on lactation potential of developing heifers. J. Dairy Sci. 81:243-249.

Park, C.S., G. M. Erickson, Y.J. Choi, and G.D. Marx. 1987. Effect of compensatory growth on regulation of growth and lactation: response of dairy heifers to a stair-step growth pattern. J. Anim. Sci. 64:1751-1758.

Petitclerc, D. P. Dumoulin, H. Ringuet, J. Matte, and C. Girard. 1999. Plane of nutrition and folic acid Suppl.ation between birth and four months of age on mammary development of dairy heifers. Can. J. Anim. Sci. 79:227-234.

Pirlo, G., M. Capelletti, and G. Marchetto. 1997. Effects of energy and protein allowances in the diets of prepubertal heifers on growth and milk production. J. Dairy Sci. 80:730-739.

Plath-Gabler, A., C. Gabler, F. Sinowatz, B. Berisha, and D. Schams. 2001. The expression of the IGF-I family and GH receptor in the bovine mammary gland. J. Endocrinol. 168:39-48.

Prosser. C.G., I. R. Fleet, A.N. Corps, E.R. Froesch, and R.B. Heap. 1990. Increase in milk secretion and mammary blood flow by intra-arterial infusion of insulin-like growth factor-I into the mammary gland of the goat. J. Endocrinol. 126:437-443.

Purup, S., K. Sejrsen, J. Foldager, and R.M. Akers. 1993. Effect of exogenous bovine growth hormone and ovariectomny on prepubertal mammary growth, serum hormones and acute in-vitro proliferative responses of mammary explants from Holstein heifers. J. Endocrinol. 139:19-26.

Purup, S., M. Vestergaard, and K. Sejrsen. 1996. The influence of feeding level and exogenous bovine somatotropin on mammary tissue sensitivity to insulin-like growth factor-I in prepubertal heifers. J. Dairy Sci. 79(Suppl. 1): 195.

Quigley, J.D., K.R. Martin, D.A. Bemis, L.N.D. Potgieter, C.R. Reinemeyer, B.W. Rohrbach, H.H. Dowlen, and K.C. Lamar. 1995. Effects of housing and colostrum feeding on serum immunoglobulins, growth, and fecal scores on Jersey calves. J. Dairy Sci. 78:893-901.

Quigley, J.D., C.A. Kost, and T.M. Anspach. 2001. Plasma IgG concentration in neonatal calves in response to a colostrum Suppl. or colostrum replacer and addition of deoxycholic acid. J. Dairy Sci. 84(Suppl. 1):224.

Radcliff, R.P., M.J. VandeHaar, A.L. Skidmore, L.T. Chapin, B.R. Radke, J.W. Lloyd, E.P. Stanisiewski, and H.A. Tucker. 1997. Effects of diet and bovine somatotropin on heifer growth and mammary development. J. Dairy Sci. 80:1996-2003.

Rauprich, A.B.E., H.M. Hammon, and J. W. Blum. 2000. Influence of feeding different amounts of first colostrums on metabolic endocrine, and health status and on growth performance in neonatal calves. J. Anim. Sci. 78:896-908.

Robinson, J.D., G.H. Stott, and S.K. DeNise. 1988. Effects of passive immunity on growth and survival in the dairy heifer. J. Dairy Sci. 71:1282-1287.

Ruan, W., and D.L. Kleinberg. 1999. Insulin-like growth factor I is essential for terminal end bud formation and ductal morphogenesis during mammary development. Endocrinol. 140:5075-5081.

Sansinanea, A.S., S.I. Cerone, I. Zonco, C.García, and N. Auza. 2001. Serum leptin levels in cattle with different nutritional conditions. Nutr. Res. 21:1045-1052.

Sarkar, N.K., G.A. Lodge, C.J. Williams, and J.I. Elliot. 1983. The effects of under nutrition of suckled pigs on subsequent growth and body composition after nutritional rehabilitation. J. Anim. Sci 57:34-42.

Schmidt, G.H. 1971. Biology of Lactation. W.H. Freeman and Company, San Francisco, CA.

Sejrsen, K. J. Foldager, and M.T. Sorensen. 1986. Effect of bovine somatotropin on pubertal mammary development in heifers. J. Dairy Sci. 69:1528-1535.

Sejrsen, K., J.T. Huber, H.A. Tucker, and R.M. Akers. 1982. Influence of nutrition on mammary development in pre and post pubertal heifers. J. Dairy Sci. 65:793-800.

Sejrsen, K., J.T. Huber, H.A. Tucker, and R.M. Akers. 1983. Influence of amount fed on hormone concentrations and their relationship to mammary growth in heifers. J. Dairy Sci. 66:845-855.

Sejrsen, K., and S. Purup. 1997. Influence of prepubertal feeding level on milk yield potential of dairy heifers: A Review. J. Anim. Sci. 75:828-835.

Sejrsen, K., S. Purup, H. Martinussen, and M. Vestergaard. 1998. Effect of feeding level on mammary gland growth in calves and prepubertal heifers. J. Dairy Sci. 81(Suppl. 1):377.

Sejrsen, K., S. Purup, M. Vestergarard, and J. Foldager. 2000. High body weight gain and reduced bovine mammary growth: physiology basis and implications for milk yield potential. Dom. Anim. Endocrinol. 19:93-104.

Silva, L.F.P., M.J. VandeHaar, and M.S. Weber Nielsen. 2002. Intramammary infusion of IGF-I increases growth of mammary epithelial cells of prepubertal heifers. J. Dairy Sci. 85:(Suppl. 1):77.

Silva, L.F.P., M.J. VandeHaar, M.S. Weber Nielsen, and B.E. Etchebarne. 2002. Leptin reduces proliferation of a bovine mammary epithelial cell line (MAC-T). J. Dairy Sci. 85:(Suppl. 1):1352.

Sinha, Y.N., and H.A. Tucker. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci. 52:507-512.

Sharma, B.K., M.J. VandeHaar, and N.K. Ames. 1994. Expression of insulin-like growth factor-I in cows at different stages of lactation and in late lactation in cows treated with somatotropin. J. Dairy Sci. 77:2232.

Smith, J.L., and L.G. Sheffield. 2002. Production and regulation of leptin in bovine mammary epithelial cells. Dom. Anim. Endocrinol. 22:145-155.

Smith, J.M., M.C. Diaz, M.E. Van Amburgh, D.E. Bauman, and M.C. Lucy. 1998. Ontogeny of the somatotropic axis in milk-fed bull calves between birth and 105-kg. Pages 119-129 *in* Proc. Cornell Nutr. Conf. for Feed Manuf., Rochester, NY. Cornell Univ., Ithaca, NY.

Spain, J.N., and D.E. Spiers. 1996. Effects of Suppl.al shade on thermoregulatory response of calves to heat challenge in a hutch environment. J. Dairy Sci. 79:639-646.

Swanson, E.W. 1960. Effect of rapid growth with fattening of dairy heifers on their lactational ability. J. Dairy Sci. 43:377-387.

Tikofsky, J. N., M. E. Van Amburgh, and D. A. Ross. 2001. Effect of varying carbohydrate and fat content of milk replacer on body composition of Holstein bull calves. J. Anim. Sci. 79:2260-2267.

Tomkins, T., J. S. Sowinski, and N.K. Keith. 1994. The influence of energy level in milk replacer on performance of male Holstein calves raised under two different environmental conditions. J. Anim. Sci. 72(Suppl. 2):49.

Tucker, H.A. 1969. Factors affecting mammary gland cell numbers. J. Dairy Sci. 52:720-729.

Van Amburgh, M.E., D.M. Galton, D.E. Bauman, R.W. Everett, D.G. Fox, L.E. Chase, and H.N. Erb. Effects of three prepubertal body growth rates on performance of Holstein heifers during first lactation. J. Dairy Sci. 81:527-538.

Vann, R.C., and J.F. Baker. 2001. Calf serum IgG affects weaning performance. J. Dairy Sci. 84(Suppl. 1):323.

Waldo, D.R., H.F. Tyrrell, A.V. Capuco, and C.E. Rexroad, Jr. Components of growth in Holstein heifers fed either alfalfa or corn silage diets to produce two daily gains. J. Dairy Sci. 80:1674-1684.

Williams, P.E.V., D. Day, and A.M. Raven. 1981. The effect of climatic housing and level of nutrition on the performance of calves. Anim. Prod. 32:133-141.

Wright, I.A., and A.J.F. Russel. 1991. Changes in the body composition of beef cattle during compensatory growth. Anim. Prod. 52:105-113.